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# Impact of Oil and Oil-Dispersant Mixtures on Freshwater Pond Ecosystems

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# Executive Summary

Oil or oil-dispersant mixtures were added to large lined freshwater ponds at oil concentrations of 100 ppm if the oil was completely dissolved. The water column biota was sampled regularly before and after treatment, as were the nutrients, dissolved oxygen and major ions. The water column biota included bacteria, fungi, phytoplankton, metazooplankton and protozooplankton. In addition, the attached material, zoobenthos, nekton and surface insects were monitored. By combining the composition and abundance of different components of the biota and the fate of the oil or oil-dispersant mixtures, it was possible to ascertain the impact of the oil-dispersant mixtures and to compare this with the impact of the oil alone. This enables those responsible for oil spill cleanups to determine whether the benefits of using a dispersant outweigh any deleterious effects.

For the first nine months following treatment, the impact of the oil and dispersant mixture on the biota was more severe than that of the oil alone. This was noted in the greater populations of heterotrophic bacteria, fungi and periphyton; the elimination of zooplankton; and the alteration of the phytoplankton. Oil as well as oil-dispersant treatments eliminated surface insects, reduced the zoobenthos and altered the protozooplankton. After the spring of the following year, the water column biota was similar in all of the ponds including the control pond. The only vestige of the impact of treatment was observed in the periphyton and zoobenthos results, which indicated that there was a residual effect by the oil remaining on the bottom.

The results show that there is a greater initial impact on the biota caused by the oil plus dispersant than by the oil alone. The results from the pond study, where the water was not exchanged, can be extrapolated to more open systems in which the influences of current, dilution and shoreline must be considered.

## Abstract

The impact of oil-dispersant mixtures on indigenous aquatic ecosystems was monitored and compared with similar systems containing oil only or left untreated. This study was carried out in large lined outdoor ponds. Sufficient oil to give 100 ppm was added, but only a small percentage of this was found in the water column. The dispersant, Corexit 9527, was initially one fifth of the oil concentration. Components of the food web investigated for this report include bacteria, fungi, phytoplankton, attached flora, zooplankton including protozoa, zoobenthos and surface insects as well as a range of water quality parameters. The oil that was in the water column or deposited on the sediment in the oil-treated pond reduced the zooplankton and protozoan populations, eliminated surface insects and affected the zoobenthos at least for the first nine months after treatment. The oil also enhanced the fungal recoveries and altered the bacterial community. Little or no effect was noticed on the phytoplankton, periphyton or the aqueous nutrients. After the spring thaw of the following year, most of the affected biological components recovered. In the oil-dispersant-treated ponds, zooplankton and surface insects were eliminated, there were pronounced shifts in the bacterial and protozoan communities, and there was an unstable phytoplankton community as well as large increases in weight of the attached material, enhancement of the geo-aquatic fungi and alteration of the zoobenthos. In addition, several of the water chemistry parameters (nitrate ion, dissolved oxygen and dissolved organic carbon) were influenced over several sampling periods by secondary effects of treatment. After one year one of the oil-dispersant-treated ponds had recovered in comparison with the control pond, and the other oil-dispersant-treated pond had nearly recovered.



## Résumé

On a étudié les effets de mélanges d'un agent de dispersion et d'hydrocarbures sur des écosystèmes aquatiques indigènes et on les a comparés aux résultats obtenus dans des écosystèmes semblables exposés à des hydrocarbures seulement ou laissés inchangés (témoins). Les tests ont été faits à l'extérieur dans de grands bassins aux parois doublés. On a ajouté suffisamment d'hydrocarbures pour que leur concentration fût de 100 ppm, mais seulement un faible pourcentage de cette concentration se retrouvait dans la colonne d'eau. Initialement, la concentration du dispersant, le Corexit 9527, représentait un cinquième de celle des hydrocarbures. Le réseau trophique étudié comprenait des bactéries, des champignons, du phytoplancton, les plantes fixées, du zooplancton (y compris des protozoaires), le zoobenthos, et des insectes de surface; on a aussi considéré divers paramètres de qualité de l'eau. Dans le bassin exposé aux hydrocarbures, ceux-ci ont réduit les populations de zooplancton et de protozoaires, éliminé les insectes de surface et eu un effet sur le zoobenthos pendant au moins les neuf premiers mois de l'expérience; de plus, la production de champignons a augmenté, et la population bactérienne a été modifiée. On a observé très peu d'effets sur le phytoplancton, le périphyton ou les éléments nutritifs de la phase aqueuse. Au printemps suivant, après le dégel, la plupart des paramètres biologiques touchés sont redevenus normaux. Dans les bassins exposés au mélange, le zooplancton et les insectes de surface ont été éliminés, il s'est produit d'énormes variations chez les populations de bactéries et de protozoaires, les populations de phytoplancton sont devenues instables, la biomasse des plantes aquatiques s'est beaucoup accrue, la production de champignons géoaquatiques a augmenté, et le zoobenthos a subi des modifications. De plus, plusieurs paramètres chimiques de l'eau (les ions nitrate, l'oxygène dissous et le carbone organique dissous) ont été modifiés pendant une période couvrant plusieurs échantillonnages par les effets secondaires du traitement. Après un an, un des bassins exposés au mélange était redevenu normal, comparativement au témoin, et l'autre était presque normal.

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# Introduction

Accidental spills of petroleum and its products in the aquatic environment occur frequently. Quite apart from ecological effects, the aesthetics of the situation necessitate the cleanup of the spilled material. Usually cleanup is restricted to mechanical methods which reduce the amount of oil in contact with the environment. Yet there are instances in which mechanical cleanup methods cannot be used, and other methods must be considered. One technique is the application of dispersants. Before such methods are contemplated, it must be shown that these methods are less deleterious to the environment than the original pollutant.

There is a large amount of material published on the effects of oil pollution on aquatic ecosystems (1,2). The reports cited in these bibliographies generally involve case histories of spills or laboratory experiments which have a limited number of biological species in each test. Intermediate to these two extremes are a smaller number of reports which consider planned spills on lakes (3,4,5) or large outdoor ponds (6,7). These experiments permit the evaluation of the aquatic community prior to any treatment and, if there are control areas, the direct comparison of the treatment with the controls. In addition, as with the case history method, a wide variety of samples can be collected over an extended period of time and analyzed by professionals in diverse fields of expertise. Such outdoor experiments experience the wide range of environmental factors that impinge on natural spills.

By using large outdoor ponds, the authors examined the effect of oil and oil-dispersant mixtures on the indigenous aquatic biota. A description of the fate and distribution of the oil and the oil-dispersant mixture has been given in an earlier report (8). In this publication, the authors will report on the bacteria, fungi, protozooplankton, mesoplankton, zoobenthos, phytoplankton and the water chemistry parameters of alkalinity, dissolved oxygen, phosphorus, nitrogen and sulphate ion.

Each major area of study is chapterized, and the names of the investigators responsible for those results are listed. The formal discussion in Chapter 6 interfaces the biological findings with the oil results and considers the areas in which the pattern of one set of results is reflected by those of another area. The appendices contain results from ancillary laboratory studies or more detailed lists of data.

## POND PREPARATION AND TREATMENT

The site selected for this study was at Baie du Doré, Ontario, opposite the Bruce power plants on Lake Huron. Construction details are given elsewhere (8). In essence, one large pond (22.5 x 9 x 2 m) was divided approximately equally into five ponds by separating each "sub-pond" from its adjacent environment with four layers of continuous 6-mil black polyethylene sheeting, including the bottoms. This gave individual self-contained ponds of about 4.5 x 9 x 2 m. A 5-cm layer of clean gravel containing silt and sand was placed over the bottom of the ponds on the liners to provide sediment. Additional gravel was placed on the sides. (The particle size distribution of the gravel is given in Appendix A.) Water from nearby Lake Huron was pumped into the ponds to a depth of 1.5 m. Adjacent ponds were separated by eight layers of polyethylene falling vertically from support beams. The polyethylene at the sides was buried in the earth and the perimeter of the ponds was mounded with gravel to limit excessive water runoff into the ponds. The only input to the ponds was from the atmosphere or in the form of runoff from rain or melting snow.

The construction of the ponds was completed in September 1977, and the ponds were left to equilibrate until January 1978. At that time, sampling was initiated on a regular basis. During periods of ice cover samples were collected once a month. When there was no ice cover, samples were collected every second week except at the time of treatment when the samples were collected one week prior to, immediately before, 24 h after, and one week after treatment. The ice cover over the ponds melted in April of 1978 and formed again in early December. Treatment occurred on July 5, 1978, when 6 L of oil was added to each of three ponds. In two ponds, the oil was pre-mixed with 1 L of dispersant and 13 L of pond water. As in previous studies (6,7), Norman Wells crude oil was used and Corexit 9527 was the dispersant; both were supplied by Imperial Oil Canada Limited. For treatment,

the oil or oil mixture was poured from a catwalk over the ponds, dropping about 1 m onto the geometric centre of the pond surface.

Ponds were assigned the numbers 1 to 5, with ponds Nos. 1 and 5 being the two exterior ponds, No. 1 being the closest to the lake. Pond No. 1 was the most westerly pond and pond No. 5, the most easterly. Pond No. 5 was shaded in the early morning by tall cedar trees. The supporting beams between the ponds caused shadows to cover one-half the bottom of the interior ponds by mid-afternoon. Ponds Nos. 1 and 3 received the oil-dispersant treatment and pond No. 4 was treated with oil only. Pond No. 2 was the control pond and pond No. 5 was left in reserve as an auxiliary control pond. In the interior treated ponds, the concentration of oil would have been 100 ppm if all of the oil had completely dissolved in the water column. For pond No. 1, because of its slightly larger volume, a theoretical value of 70 ppm would have been expected. The theoretical concentration of the dispersant in pond No. 3 would have been 20 ppm if it had completely dissolved and in pond No. 1, 15 ppm.

# Phytoplankton, Periphyton and Water Chemistry

*by Brian F. Scott and Valanne Glooschenko*

## METHODS

During periods of ice cover, a clear 2-L Plexiglas Van Dorn sampler was utilized to collect water samples from just below the ice surface, at mid-depth and just above the bottom. These samples were combined into a composite sample, of which 1 L was placed in a storage bottle containing modified Lugol's solution (5 mL). During periods of open water, four sampling locations in each pond were used, and the samples were collected from near surface and near bottom, resulting in eight discrete contributions to the composite sample. On two occasions, one at 56 days after treatment and the other at 356 days after treatment, the eight subsamples were analyzed individually to ascertain the patchiness or heterogeneity of the phytoplankton in the ponds. All treated samples were stored in a dark cool (4°C) room.

At the time of analysis, the sample bottle was gently shaken and an aliquot of the contents was put into a 50-mL Ütermohl settling cylinder which was placed over an Ütermohl counting chamber. After sufficient time for settling, the cylinder was removed and the counting chamber was placed on a Leitz "Divert" inverted microscope; then x 400 magnification was used for enumeration. Organisms greater than 5 μm were counted in several 0.25-mm strips; smaller organisms were counted over a smaller area. Between 200 and 400 cells were counted for each sample and a replicate was performed on at least one sample for each sampling date. These counts were reported as the number of organisms per litre. Average cell volumes were recorded for each species and these values were converted to biomass, assuming a density of 1 g/mL (9). Smaller algae were identified by x 1000 magnification under oil. Standard taxonomic sources (10,11,12,13,14,15,16,17,18) were used.

Periphyton was collected from blasted Plexiglas plates. These plates, measuring 7.5 x 7.5 x 0.5 cm, were anchored vertically on weighted platforms which rested on the bottoms of the ponds. During the ice-free period, the platforms were raised once a month and a minimum of two plates was removed. With the aid of toothbrushes and distilled water, material was brushed into small 250-mL bottles which contained 3 mL of modified Lugol's solution and then stored, as was the phytoplankton until analysis. Immediately before analysis, the contents were placed in a blender and homogenized; then the material was returned to the bottle and the volume was adjusted to 250 mL. A known aliquot was then filtered through predried and weighed Whatman GF/C filter paper. The filter paper was dried overnight at 85°C for the determination of the dry weight and then heated for two more hours at 500°C to obtain the organic carbon free (OCF) weight.

Water samples for chemical analysis were taken from the composite samples, treated at the site and returned to the laboratories on ice where they were analyzed for $NH_3$, $NO_2$, $NO_3^-$, total Kjeldahl nitrogen (TKN), unfiltered and filtered P, dissolved reactive P, dissolved reactive silica, alkalinity, dissolved organic carbon (DOC), $SO_4^-$, $Cl^-$, $Ca^{++}$ and $Mg^{++}$ using standard methods (19). Snow samples from the immediate area were melted in clean vessels and treated like water samples. Aliquots for chlorophyll analysis were taken from the composite, and a known volume was passed through Whatman GF/C glass microfibre filters, with the residue on the filter paper being kept away from light, and returned to the laboratories on ice. They were stored at -20°C until analyzed. When the eight subsamples were collected and stored separately on August 29, 1978, for phytoplankton analysis, additional water was removed from each of the eight subsamples from ponds Nos. 1 and 2 for chlorophyll analysis. Chlorophyll content of the material on the filter paper was determined using a method recommended by Burnison (20). Dissolved oxygen concentrations were determined *in situ* using a Yellow Springs Instrument Company oxygen meter equipped with a probe. Readings were taken just under the surface, at mid-depth and near the sediment.

## RESULTS

### *Phytoplankton*

Prior to treatment, from spring to early summer of 1978, all of the ponds had significant contributions to the phytoplankton from Chrysophyceae, Cryptophyceae, Chlorophyceae and Dinophyceae, as shown in Figures 1, 2, 3 and 4 for ponds Nos. 1, 2, 3 and 4, respectively. Lesser amounts of Cyanophyceae and Bacillariophyceae (diatoms) were also noted. Ponds Nos. 2, 3 and 4 experienced a

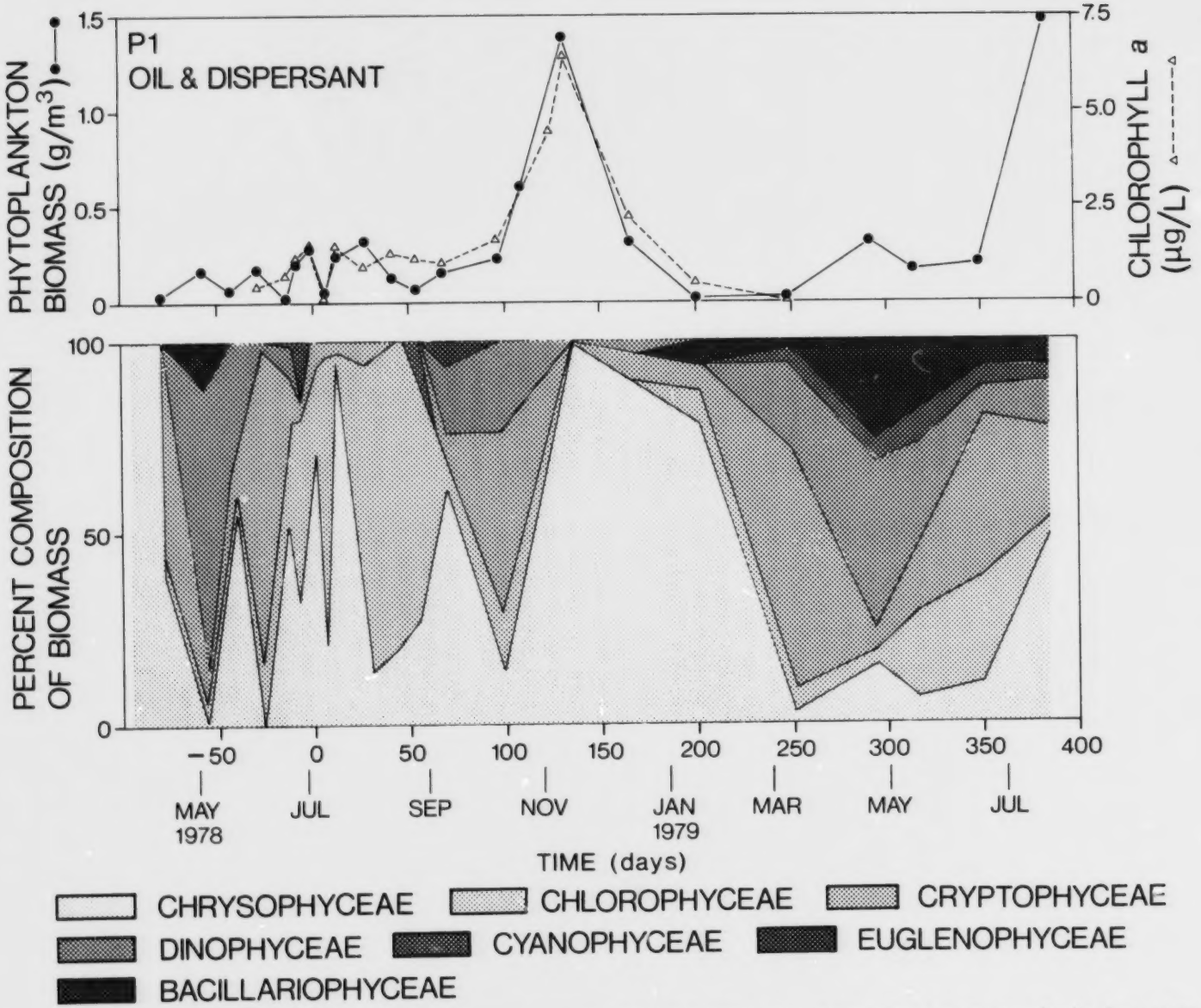



**Figure 1.** Phytoplankton results for pond No. 1 (oil and dispersant treatment). The lower diagram shows the percentage contribution of major classes to the biomass. The upper diagram indicates the phytoplankton biomass and the chlorophyll *a* values.

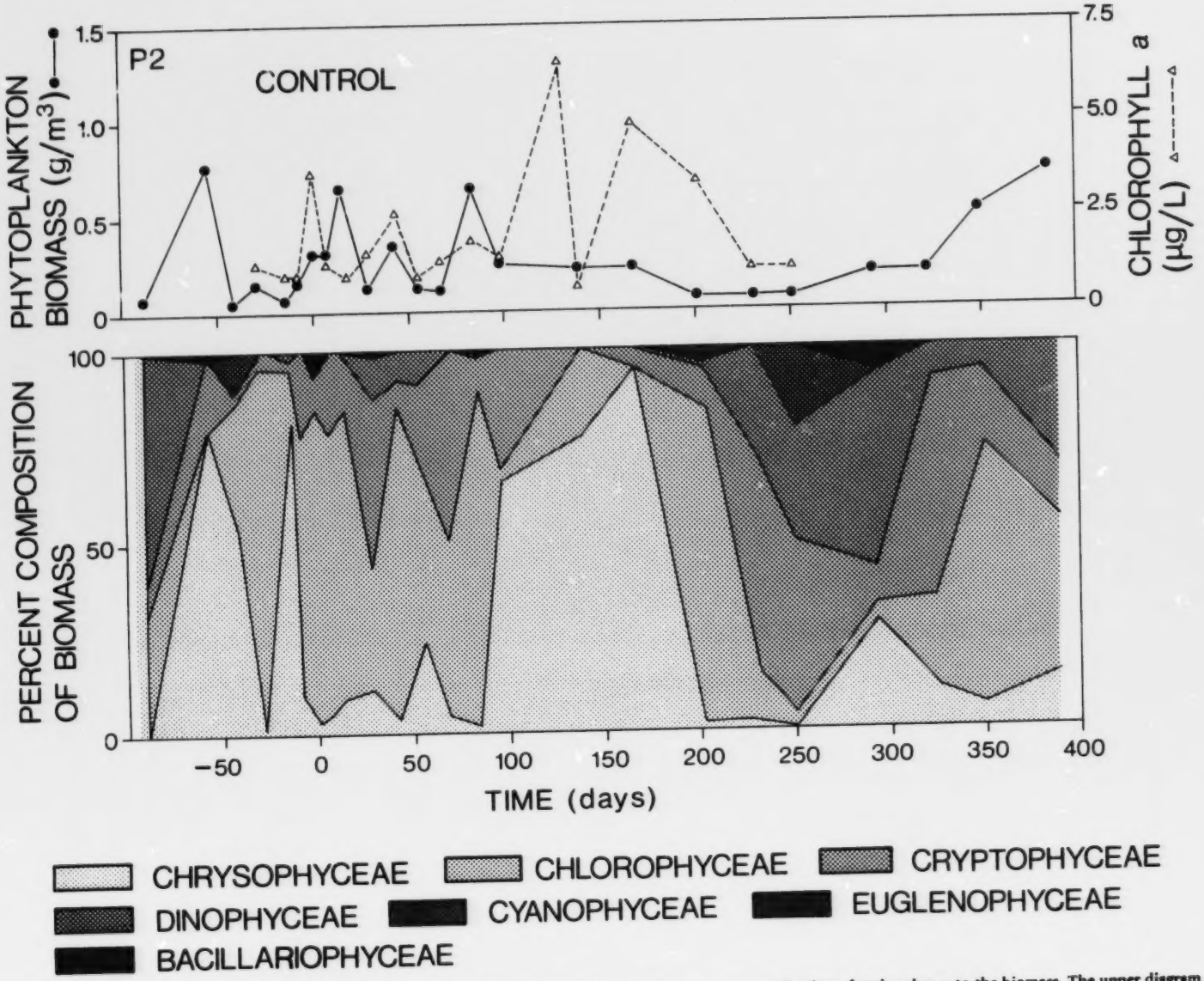



**Figure 2.** Phytoplankton results for pond No. 2 (control). The lower diagram shows the percentage contribution of major classes to the biomass. The upper diagram indicates the phytoplankton biomass and the chlorophyll *a* values.

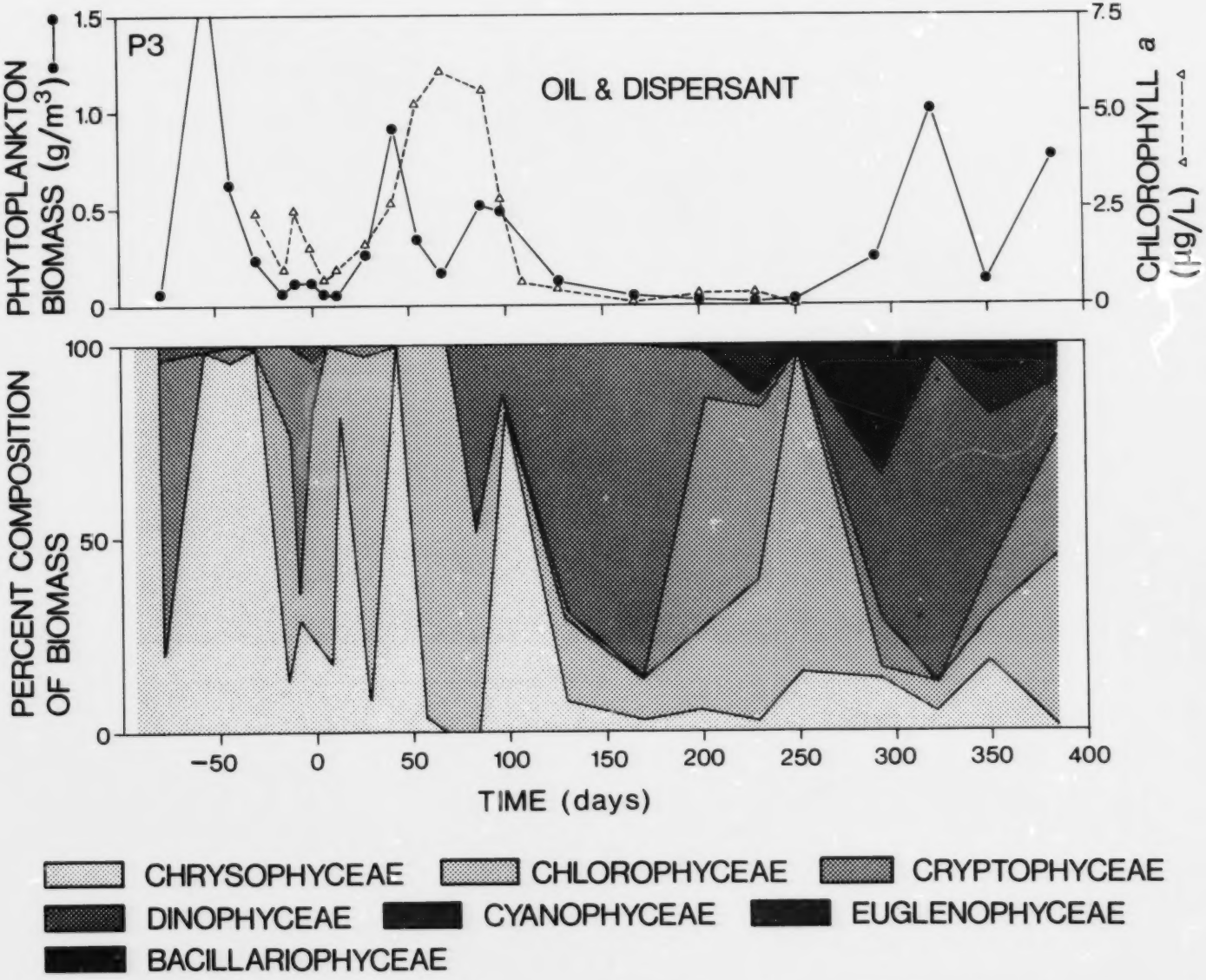



**Figure 3.** Phytoplankton results for pond No. 3 (oil and dispersant treatment). The lower diagram shows the percentage contribution of major classes to the biomass. The upper diagram indicates the phytoplankton biomass and the chlorophyll *a* values.

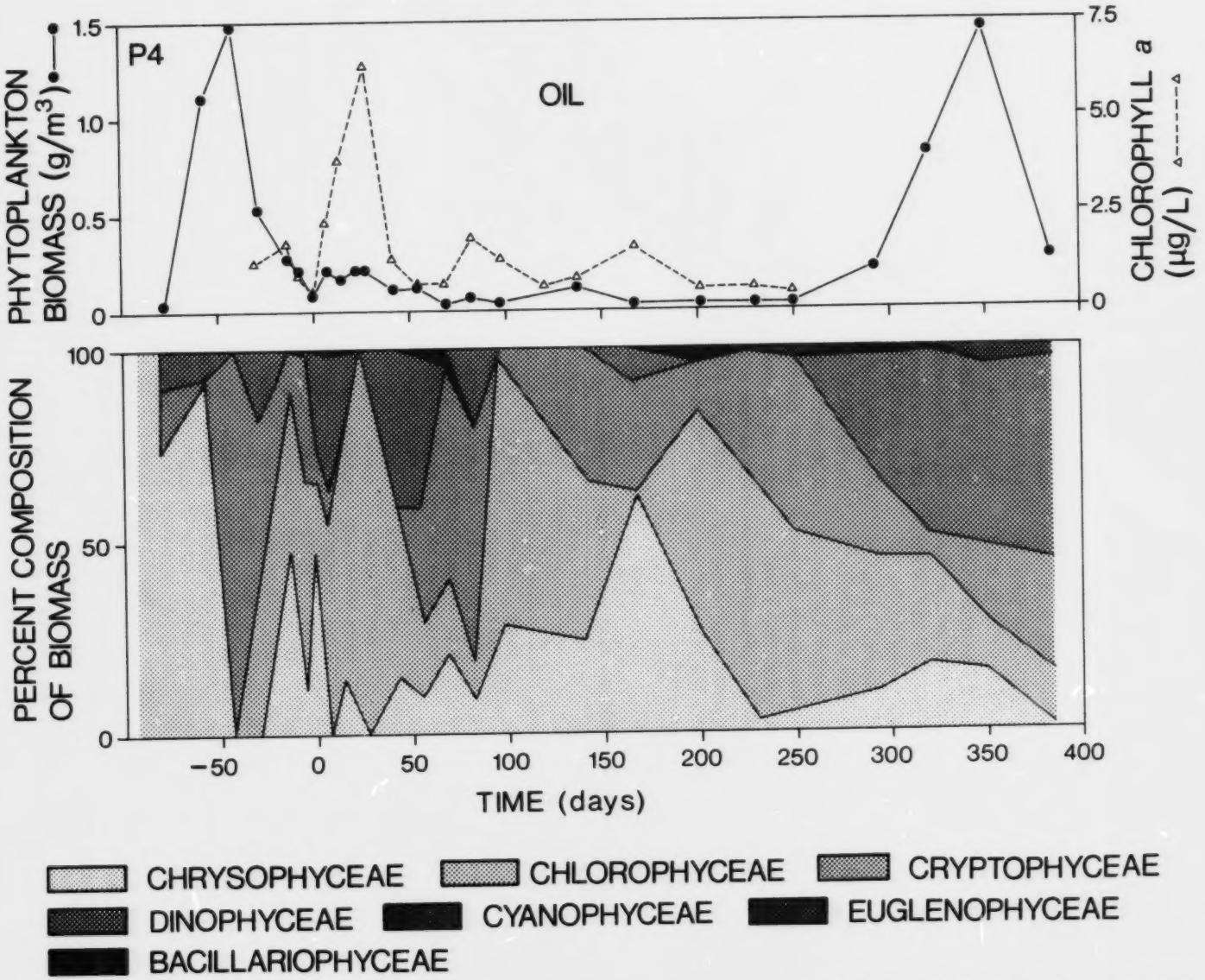



**Figure 4.** Phytoplankton results for pond No. 4 (oil treatment). The lower diagram shows the percentage contribution of major classes to the biomass. The upper diagram indicates the phytoplankton biomass and the chlorophyll *a* values.

phytoplankton bloom of *Dinobryon* sp. in the early spring. Each pond, once past the period of the spring bloom, had at least two algal phyla or classes contributing to the phytoplankton biomass on any sampling date. The control and oil-treated ponds exhibited this general trend, as shown in Figures 2 and 4, until the end of the experiment, indicating that the amount of oil added to pond No. 4 had little effect on the phytoplankton. After the additions of the oil-dispersant mixtures to ponds Nos. 1 and 3, there was a trend for one of the classes to dominate the phytoplankton. Generally, a single genus dominated, although the same genus was not necessarily present in both of these ponds. One week after treatment the biomass in both oil-dispersant-treated ponds decreased. *Ochromonas* spp. dominated the biomass in these two ponds on day 14 and *Pandorina morum* dominated in both ponds on day 28. On the next sampling date (day 41), *Öocystis submarina* dominated in pond No. 1 and *Ochromonas*, in pond No. 3. At the end of August, 55 days after treatment, *Chlorella* spp. dominated the phytoplankton in both ponds. On the next sampling date, September 12, pond No. 1 had a number of classes contributing to the phytoplankton biomass, but pond No. 3, the interior pond, was dominated by *Öocystis* sp. After this time both ponds usually had several taxa contributing to the phytoplankton, although there were occasions when there was only one taxon dominating the biomass during October and November of 1978. Appendix B lists the major contributors to the phytoplankton in each pond during the experiment.

To ascertain the degree of patchiness of the phytoplankton, the eight subsamples that comprise the composite sample were analyzed individually on two occasions. The first set of samples analyzed in this way was taken August 29, 1978, and the results are shown in Table 1a. These results show that for the control and oil-treated ponds, there is a trend toward patchiness, particularly in pond No. 4. *Öocystis lacustris* dominated the Chlorophyceae in six of the seven subsamples analyzed for pond No. 4 and *Peridinium pulvisculus* dominated its taxon in the samples that contained this dinoflagellate. The range of the total phytoplankton biomass for pond No. 4 was from 0.042 to 0.26 g/m$^3$, these values differing by a factor of 6. The biomass depended on sample location, but these changes did not depend on whether the sample was taken from near surface or near bottom. The samples from the control pond (pond No. 2) exhibit more uniformity. In this pond the Chlorophyceae were usually the dominant class in all

**Table 1a. Analysis of Composite Samples, August 29, 1978**

| Pond No. | Parameters | Subsample position number | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | Chlorophyceae | 0.050 | 0.040 | 0.067 | 0.054 | 0.051 | 0.054 | 0.045 | 0.047 |
| | Chrysophyceae | 0.013 | 0.018 | 0.027 | 0.042 | 0.016 | 0.021 | 0.015 | 0.016 |
| | Total biomass | 0.063 | 0.058 | 0.094 | 0.096 | 0.067 | 0.075 | 0.070 | 0.063 |
| | Cells per litre X $10^{-6}$ | 8.82 | 6.23 | 13.4 | 10.02 | 7.71 | 8.35 | 7.36 | 8.90 |
| | Chlorophyll *a* (μg/L) | 1.05 | 1.42 | 2.21 | 1.78 | 1.11 | 1.59 | 1.20 | 2.25 |
| 2 | Chlorophyceae | 0.195 | 0.023 | 0.045 | 0.022 | 0.132 | 0.008 | 0.048 | 0.004 |
| | Chrysophyceae | 0.035 | 0.049 | 0.003 | 0.020 | 0.009 | 0.007 | 0.019 | 0.041 |
| | Cryptophyceae | 0.024 | 0.033 | 0.040 | 0.045 | 0.007 | 0.002 | 0.018 | 0.032 |
| | Dinophyceae | 0.004 | 0.008 | 0.043 | 0.011 | 0.004 | 0.002 | – | – |
| | Total biomass | 0.258 | 0.113 | 0.131 | 0.098 | 0.152 | 0.019 | 0.085 | 0.077 |
| | Cells per litre X $10^{-6}$ | 0.718 | 0.332 | 0.742 | 0.509 | 0.644 | 0.543 | 1.11 | 1.28 |
| | Chlorophyll *a* (μg/L) | 0.96 | 0.69 | 1.46 | 0.42 | 0.14 | 0.90 | 0.90 | 0.76 |
| 3 | Chlorophyceae | 0.321 | 0.220 | 0.352 | 0.428 | 0.303 | 0.323 | 0.368 | 0.323 |
| | Chrysophyceae | 0.018 | 0.028 | 0.015 | 0.010 | 0.009 | 0.023 | 0.011 | 0.013 |
| | Total biomass | 0.339 | 0.248 | 0.367 | 0.438 | 0.312 | 0.346 | 0.377 | 0.336 |
| | Cells per litre X $10^{-6}$ | 43.7 | 35.5 | 17.4 | 21.4 | 15.0 | 16.2 | 16.2 | 18.4 |
| 4 | Chlorophyceae | 0.007 | 0.027 | 0.033 | 0.015 | 0.013 | 0.004 | | 0.092 |
| | Chrysophyceae | – | 0.004 | 0.010 | 0.005 | 0.005 | 0.011 | | 0.012 |
| | Cryptophyceae | 0.022 | 0.009 | 0.017 | 0.038 | 0.025 | 0.018 | | 0.027 |
| | Dinophyceae | 0.012 | 0.052 | 0.081 | – | 0.009 | 0.075 | | 0.132 |
| | Bacillariophyceae | – | 0.002 | 0.002 | 0.001 | – | 0.002 | | – |
| | Cyanophyceae | 0.001 | – | – | – | – | 0.020 | | – |
| | Total biomass | 0.042 | 0.094 | 0.143 | 0.059 | 0.052 | 0.130 | | 0.263 |
| | Cells per litre X $10^{-6}$ | 1.77 | 0.519 | 0.497 | 0.859 | 0.630 | 2.27 | | 1.11 |

Note: Parameters are in grams per cubic metre unless indicated otherwise.

of the subsamples. The biomass had a range of 0.08 to 0.26, differing by a factor of 3. At the time of sampling, both ponds had a low turbidity with the bottom of the ponds being clearly visible. The samples were taken under a bright morning sun. Results derived from the subsamples from the two oil-dispersant-treated ponds (ponds Nos. 1 and 3) showed a high degree of uniformity, but there were only Chlorophyceae and Chrysophyceae in these two ponds, with Chlorophyceae dominating. The extreme high and low biomass values calculated for the samples from these two ponds differ by a factor of less than two, while pond No. 3 had a biomass four times greater than that of pond No. 1. The Secchi depths in these two ponds were estimated to be less than 10 cm.

The results from the July 25, 1979 subsamples are listed in Table 1b. These results show a similarity for all of the subsamples in their percent composition of contributing classes and total biomass, with the average agreeing with the composite values. In addition, the same species of a particular class dominates in all of the subsamples and the composite. These data are tabulated in Appendix C for the subsamples and the composite is tabulated in Appendix B. This sampling occurred on a sunny day and all of the pond bottoms were visible.

On August 29, 1978, there may have been little mixing in the water column resulting in heterogeneity of the phytoplankton in ponds Nos. 2 and 4. The agreement between values from the subsamples in the two oil-dispersant-treated ponds may in part have resulted from some factor associated with the high turbidity of the water in these two ponds. The results of the second intensive analysis indicate that the ponds were well mixed, with the effect of the oil and oil-dispersant mixture on the phytoplankton being virtually nonexistent at that time.

**Table 1b. Analysis of Composite Samples, July 25, 1979**

| Pond No. | Parameters | Subsample position number | | | | | | | | Composite |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| 1 | Chlorophyceae | 0.011 | 0.008 | 0.009 | 0.004 | 0.009 | 0.008 | 0.006 | 0.010 | 0.003 |
| | Chrysophyceae | 0.061 | 0.059 | 0.077 | 0.070 | 0.050 | 0.057 | 0.050 | 0.077 | 0.074 |
| | Cryptophyceae | 0.035 | 0.049 | 0.035 | 0.048 | 0.026 | 0.051 | 0.030 | 0.043 | 0.038 |
| | Dinophyceae | 0.034 | 0.040 | 0.048 | 0.049 | 0.027 | 0.030 | 0.026 | 0.050 | 0.018 |
| | Bacillariophyceae | 0.010 | 0.008 | 0.009 | 0.009 | 0.009 | 0.008 | 0.009 | 0.001 | 0.009 |
| | Cyanophyceae | 0.004 | 0.002 | 0.003 | 0.004 | 0.006 | 0.008 | 0.002 | 0.000 | 0.006 |
| | Total biomass | 0.155 | 0.166 | 0.181 | 0.284 | 0.134 | 0.162 | 0.123 | 0.181 | 0.148 |
| | Cells per litre X $10^{-6}$ | 7.16 | 7.23 | 9.07 | 8.97 | 5.78 | 7.43 | 6.52 | 9.45 | 8.74 |
| 2 | Chlorophyceae | 0.026 | 0.026 | 0.021 | 0.023 | 0.015 | 0.026 | 0.023 | 0.022 | 0.029 |
| | Chrysophyceae | 0.008 | 0.009 | 0.007 | 0.006 | 0.001 | 0.005 | 0.010 | 0.009 | 0.010 |
| | Cryptophyceae | 0.014 | 0.014 | 0.015 | 0.019 | 0.009 | 0.013 | 0.023 | 0.017 | 0.021 |
| | Dinophyceae | 0.047 | 0.029 | 0.051 | 0.018 | 0.038 | 0.022 | 0.010 | 0.016 | 0.021 |
| | Bacillariophyceae | – | – | – | – | – | 0.000 | 0.000 | 0.000 | – |
| | Cyanophyceae | – | – | – | – | – | – | – | – | – |
| | Total biomass | 0.095 | 0.078 | 0.095 | 0.066 | 0.63 | 0.067 | 0.066 | 0.064 | 0.072 |
| | Cells per litre X $10^{-6}$ | 7.45 | 6.38 | 6.05 | 6.89 | 1.70 | 7.49 | 7.57 | 7.14 | 8.50 |
| 3 | Chlorophyceae | 0.031 | 0.031 | 0.035 | 0.038 | 0.037 | 0.040 | 0.039 | 0.039 | 0.035 |
| | Chrysophyceae | – | – | 0.001 | 0.001 | 0.001 | – | 0.001 | – | 0.001 |
| | Cryptophyceae | 0.016 | 0.031 | 0.015 | 0.012 | 0.010 | 0.041 | 0.013 | 0.020 | 0.023 |
| | Dinophyceae | 0.008 | 0.007 | 0.008 | 0.008 | 0.009 | 0.005 | 0.018 | 0.003 | 0.010 |
| | Bacillariophyceae | 0.003 | 0.005 | 0.003 | 0.005 | 0.005 | 0.003 | 0.002 | 0.004 | 0.005 |
| | Cyanophyceae | 0.002 | 0.003 | 0.001 | 0.003 | 0.001 | 0.002 | 0.002 | 0.005 | 0.003 |
| | Total biomass | 0.060 | 0.077 | 0.058 | 0.067 | 0.063 | 0.091 | 0.075 | 0.071 | 0.077 |
| | Cells per litre X $10^{-6}$ | 1.57 | 1.60 | 1.58 | 1.98 | 1.62 | 1.94 | 1.86 | 1.98 | 1.77 |
| 4 | Chlorophyceae | 0.031 | 0.031 | 0.035 | 0.038 | 0.037 | 0.040 | 0.039 | 0.039 | 0.034 |
| | Chrysophyceae | 0.001 | – | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | – | 0.001 |
| | Cryptophyceae | 0.016 | 0.031 | 0.015 | 0.013 | 0.010 | 0.041 | 0.013 | 0.020 | 0.023 |
| | Dinophyceae | 0.008 | 0.007 | 0.008 | 0.008 | 0.009 | 0.005 | 0.018 | 0.003 | 0.010 |
| | Cyanophyceae | 0.002 | 0.002 | 0.001 | 0.003 | 0.001 | 0.002 | 0.002 | 0.005 | 0.003 |
| | Total biomass | 0.058 | 0.071 | 0.060 | 0.063 | 0.058 | 0.089 | 0.073 | 0.067 | 0.071 |
| | Cells per litre X $10^{-6}$ | 1.57 | 1.60 | 1.58 | 1.98 | 1.62 | 1.94 | 1.86 | 1.98 | 1.77 |

**Note: Parameters are in grams per cubic metre unless indicated otherwise.**

**Table 2. Weight of Periphyton (g/m²)**

| Date | Day | Pond No. 1 | | Pond No. 2 | | Pond No. 3 | | Pond No. 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry weight | OCF weight* | Dry weight | OCF weight | Dry weight | OCF weight | Dry weight | OCF weight |
| 78-06-28 | -7 | | | 20.78(1)† | 5.62 | 7.08(1) | 3.68 | 9.33(1) | 4.40 |
| 78-08-02 | 28 | 14.42(1) | 4.94 | 10.79(1) | 4.87 | 27.77(3) | 13.92 | | |
| 78-08-29 | 55 | 89.53(3) | 61.57 | 21.09(3) | 9.53 | 39.03(3) | 30.77 | 14.3 (3) | 8.09 |
| 78-09-26 | 83 | 642.1 (3) | 77.9 | 54.25(3) | 12.18 | 123.1 (3) | 61.4 | 29.96(3) | 15.58 |
| 78-10-24 | 111 | 708.5 (3) | 90.6 | 48.33(3) | 17.82 | 183.2 (3) | 58.6 | 40.36(3) | 12.84 |
| 78-11-21 | 139 | 927.2 (3) | 134.4 | 52.82(3) | 19.37 | 224.5 (3) | 86.6 | 23.79(3) | 11.88 |
| 79-03-13 | 251 | 647.5 (1) | 98.1 | 59.65(2) | 22.9 | 206.5 (2) | 108.5 | 65.26(2) | 35.92 |
| 79-05-20 | 351 | 120.0 (1) | 28.8 | 112.2 (2) | 44.7 | 365.4 (2) | 147.5 | 75.88(2) | 33.2 |
| 79-07-24 | 385 | 64.2 (2) | 32.8 | 68.2 (2) | 22.1 | 229.2 (2) | 105.1 | 67.1 (2) | 33.6 |

*Organic carbon free weight derived by subtracting the ashed weight from the dry weight.
†Numbers in parentheses denote the number of plates analyzed.



**Table 3a. Algal Composition of Attached Material on Plates**

| Date | Pond No. 1 | Pond No. 2 | Pond No. 3 | Pond No. 4 |
|---|---|---|---|---|
| 78-06-28 | *Mougeotia* sp.<br>*Oedogonium* sp.<br>*Cosmarium punctalatum*<br>+ 13 minor species | | *Mougeotia* sp.<br>*Öocystis* sp.<br>*Oedogonium* sp.<br>+ 16 minor species | |
| 78-08-02 | | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*C. punctalatum*<br>+ 20 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>+ 5 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>+ 11 minor species |
| 78-08-29 | *Mougeotia* sp.<br>*Oedogonium* sp.<br>+ 5 minor species | *Öocystis* sp.<br>*Oedogonium* sp.<br>*Mougeotia* sp.<br>+ 25 minor species | *Mougeotia* sp.<br>+ 4 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*Achnanthes minutissima*<br>+ 28 minor species |
| 78-09-26 | *Mougeotia* sp.<br>*Oedogonium* sp.<br>+ 4 minor species | | *Mougeotia* sp.<br>*Geminella* sp.<br>+ 6 minor species | *Mougeotia* sp.<br>*Oedogonium* sp.<br>+ 17 minor species |
| 78-10-24 | *Mougeotia* sp.<br>*Oedogonium* sp.<br>+ 7 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*Öocystis* sp.<br>*Chlorococcum humicola*<br>+ 29 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*Oscillatoria limnetica*<br>+ 9 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*C. punctalatum*<br>+ 16 minor species |
| 78-11-21 | *Mougeotia* sp.<br>*Geminella mutabilis*<br>+ 7 minor species | *Mougeotia* sp.<br>*Öocystis* sp.<br>+ 20 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*O. limnetica*<br>+ 5 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*Öocystis borgei*<br>*Cyclotella* sp.<br>+ 29 minor species |
| 79-03-13 | *Mougeotia* sp.<br>*Navicula capititata*<br>+ 3 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*Synedra ulna*<br>+ 23 minor species | *Mougeotia* sp.<br>+ 7 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>*Öocystis solitaria*<br>+ 19 minor species |
| 79-05-23 | *Oedogonium* sp.<br>*Oscillatoria* sp.<br>*Mougeotia* sp.<br>+ 8 minor species | *Oedogonium* sp.<br>*Mougeotia* sp.<br>+ 19 minor species | *Oedogonium* sp.<br>*Oscillatoria* sp.<br>+ 6 minor species | |
| 79-07-24 | *Oedogonium* sp.<br>*Gleolocystis vesiculsus*<br>*Mougeotia* sp.<br>+ 12 minor species | *Oedogonium* sp.<br>*Bulbochaete*<br>+ 12 minor species | *Oscillatoria* sp.<br>*Oedogonium* sp.<br>*Mougeotia* sp.<br>+ 6 minor species | *Oedogonium* sp.<br>*Cylindrocapsa* sp.<br>+ 11 minor species |

**Table 3b. Algal Composition of Attached Material on Liner**

| Date | Pond No. 1 | Pond No. 2 | Pond No. 3 | Pond No. 4 |
|---|---|---|---|---|
| 78-04-26 | *Oedogonium* sp.*<br>*Mougeotia viridis*<br>*Pediastrum boryanum*<br>*Oocystis lacustris*<br>*Mesotaenium* sp.<br>*Achnanthes* sp. | *Oedogonium* sp.<br>*Crucigenia rectangularis* | *Ulothrix aequalis*<br>*Oedogonium* sp.<br>*Cosmarium* sp. | |
| 78-08-29 | *M. viridis**<br>*U. aequalis*<br>*Cosmarium margaritatum*<br>*O. lacustris*<br>*Oedogonium* sp.<br>*S. quadricauda*<br>*P. boryanum*<br>*Mesotaenium* sp.<br>*Chroococcus varius*<br>*Merismopedia punctata* | | *M. viridis*<br>*Microspora* sp.<br>*Oedogonium* sp.<br>*Scenedesmus quadricauda*<br>*Cosmarium* sp. | |
| 78-10-24 | *Oedogonium* sp.<br>*Chladophora* sp.<br>*U. aequalis*<br>*Achnanthes* spp.<br>*Oscillatoris* spp. | *C. rectangularis*<br>*S. quadricauda*<br>*Cosmarium margaritatum* | *U. aequalis*<br>*Oedogonium* sp. | *Microspora* sp.<br>*Oedogonium* sp.<br>*Ankistrodesmus falcatus*<br>*O. lacustris* |
| 78-11-11 | *M. viridis**<br>*Oedogonium* sp.<br>Fungal hypea | *Oedogonium* sp.*<br>*U. aequalis*<br>*M. viridis* | *Oedogonium* sp.*<br>*U. aequalis* | *Oedogonium* sp.*<br>*U. aequalis*<br>*Cosmarium* sp. |

*Denotes major contributor (greater than 80%).

Figures 1, 2, 3 and 4 also show the biomass of the phytoplankton over the time of the experiment and the chlorophyll *a* values for the period slightly prior to treatment until the end of March in the following year. In pond No. 1, the chlorophyll *a* values generally follow the trends derived from the biomass calculations. There was no correlation between these two parameters derived from the other three ponds. Table 1a lists the chlorophyll *a* values for the subsamples taken from ponds Nos. 1 and 2. When these are compared with the biomass values for the subsamples, no firm conclusions can be reached.

*Periphyton*

Periphyton results are presented in Table 2, which lists the dry weights and the OCF weights. Samples taken prior to treatment had approximately the same OCF weights, and this was also observed for the sample taken 28 days after treatment. On day 55 after treatment, the values for OCF weights were three times higher for pond No. 3 than for ponds Nos. 2 and 4. The OCF weight for pond No. 1, the other oil-dispersant-treated pond, was two times greater than that calculated for pond No. 3 at this sampling time. Throughout the rest of 1978, the weights determined for pond No. 1 were higher than those from pond No. 3, which were greater than those values determined for the control and oil-treated ponds. In March of 1979, the OCF weight from pond No. 1 was less than that determined for pond No. 3, and by July, the values from pond No. 1 were similar to those of the control and oil-treated ponds. The dry weight and OCF weights from pond No. 3 remained high.

The periphyton plates removed from all of the ponds one week before treatment and four weeks after treatment were lightly covered with light brown material. This colour of the periphyton on the plates removed from the control and oil-treated ponds persisted for the duration of the experiment. Plates removed from the oil-dispersant-treated ponds in 1978 after treatment were covered by a thick green filamentous material. In March of 1979, the platforms supporting the plates were raised to the under-ice surfaces to remove the plates. The platforms and the plates in ponds Nos. 2 and 4 appeared normal, but the platforms and plates from ponds Nos. 1 and 3 were covered with a thick gelatinous material. This material was black in pond No. 1. In pond No. 3, one half of the platform and the plates in that area were black and the other half, a light green. When the black plates were examined the next day in the laboratories, the black colour had changed to the light green found in pond No. 3, indicating the presence of $Fe^{+2}$ ion. Microscopic examination of the material revealed that it was composed of attached algae and fungi, in approximately equal amounts.

The major algal contributors to the periphyton are listed in Tables 3a and 3b along with the number of minor

contributors. The samples taken in early August showed there were many genera present in the periphyton on the plates in all of the ponds, but after that time only the oil-treated and control ponds exhibited a high number of minor contributing species. From late August until March 1979, *Mougeotia* sp. was recovered from the oil-dispersant-treated ponds with *Oedogonium* sp. being a frequent major contributor in pond No. 3. *Öocystis* and *Oedogonium* usually dominated the periphyton collected from the control and oil-treated ponds.

As the liners could easily be damaged, very few samples were collected from them. In May, June and July of 1979, scrapings from the liners were taken, and the estimated results are shown in Table 4 (8). Entries are recorded for the oil-dispersant-treated ponds at the sampling times listed above, as there was sufficient material to collect. The control pond had only one sample removed since there was only one time when there was sufficient growth for a sample to be removed. No samples were collected from the oil-treated pond. In August and September of 1978, some filamentous material was found growing in discrete bands on the liners of the oil-treated pond under the water surface. These bands were at the lower water levels that were reached during a long rain-free period. September rains raised the water levels over small amounts of "beached" oil deposited on the liners, and filamentous blue-green algae then grew on these deposits.

**Table 4. Estimated Weights of Attached Material on Pond Liners**

| Date | Pond | Estimated weights per pond dry weight (g) |
|---|---|---|
| 79-05-23 (322)* | 1 | 3 283 |
| | 3 | 7 256 |
| 79-06-19 (349) | 1 | 8 326 |
| | 2 (control) | 822 |
| | 3 | 4 255 |
| 79-07-25 (384) | 1 | 56 900 |
| | 3 | 55 600 |

*Day after treatment is given in parentheses.

### *Water Chemistry*

The dissolved oxygen readings taken from mid-depth are plotted against time in Figure 5. There were no apparent trends in these readings prior to treatment. One week after treatment both oil-dispersant-treated ponds experienced an oxygen depletion which lasted for approximately two weeks. Eight weeks after treatment, the oil-dispersant-treated ponds had dissolved oxygen values greater than those of the control or oil-treated ponds. After 16 weeks this effect was no longer apparent. The results of the diel (24 h) measurements taken eight weeks after treatment are presented in Figure 6. This diagram shows that values in the control and oil-treated ponds were lower than in the oil-dispersant-treated ponds in the afternoon and evening. At night and in the morning, the oil-dispersant-treated ponds had the lower values. This suggests that the oxygen regime at this time was dominated by the large amounts of periphytic material in the oil-dispersant-treated ponds, which produces oxygen while there is daylight but consumes oxygen at night during respiration. At about the same time while the periphyton was becoming established, the oxygen levels in the oil-dispersant ponds increased. The phytoplankton levels in all of the ponds were of the same order of magnitude after the treatment.

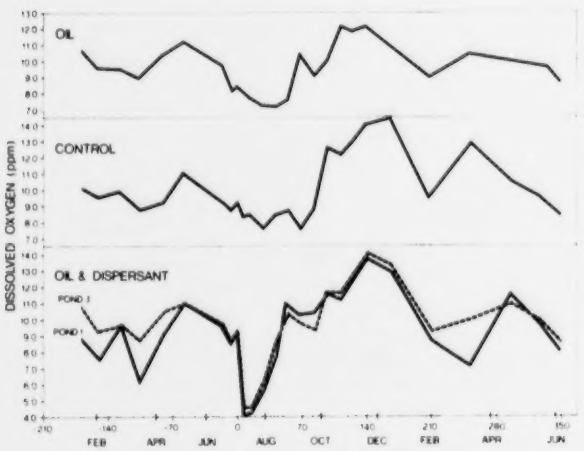



**Figure 5. Dissolved oxygen concentrations.**

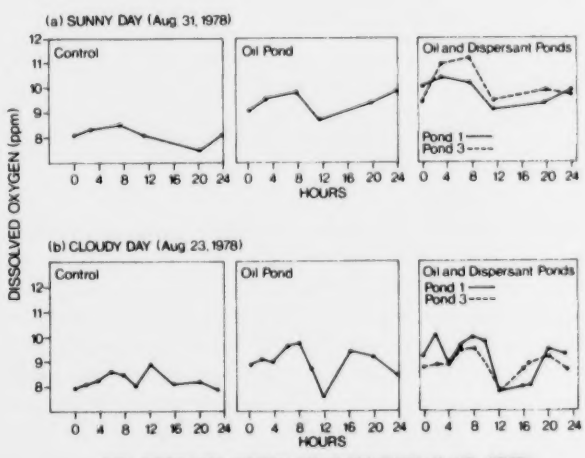



**Figure 6. Diel (24-hour cycle) oxygen concentrations on (a) a sunny day and (b) a cloudy day.**

Oxygen readings taken from the near sediment of the ponds during the winter of 1978-79 indicated a reduction in dissolved oxygen as the winter progressed. In March of 1979, this was more apparent in the two ponds treated with the oil-dispersant mixture, where the oxygen depleted zone extended farther into the water column from the bottom. Although the probe was not calibrated for anoxic conditions, the DO values read directly from the meter indicated that there were anoxic conditions within 10 cm of the bottom in pond No. 1. In pond No. 3, there were two distinct bottom levels, differing by 15 cm. Dissolved oxygen values in the deeper half of the pond were lower near the sediment than readings from near sediment of the upper level. The periphyton sampling platform was resting on the slope between these two levels before the March sampling. Ponds Nos. 2 and 4 had narrower zones of oxygen depletion (measuring up from the sediment) than the other two ponds. Total alkalinity values are plotted in Figure 7 and show no trends dependent on treatment.

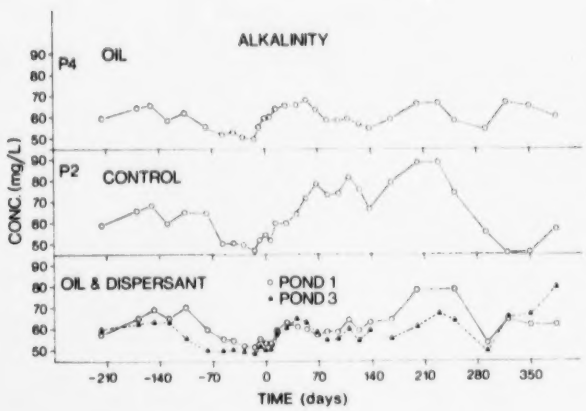



**Figure 7. Alkalinity measurements.**

Figure 8 shows the concentration of DOC as a function of time in all of the ponds. There was a low level of DOC in all of the ponds before treatment, and the levels remained low in the control pond after treatment. The DOC values were slightly higher in the oil-treated pond, but they were the highest in the oil-dispersant-treated ponds. Differences between values for ponds Nos. 1 and 3 reflect the greater volume of water present in pond No. 1, with equal amounts of the chemicals being added to both ponds. The DOC values in the oil-dispersant ponds declined about day 70, remained constant over the winter months at about 7 ppm and then diminished to about 5 ppm. This trend parallels the recovery of the aquatic biota. The DOC levels in ponds Nos. 1 and 3 were still high relative to the other ponds one year after treatment. Oil concentrations in the water column showed that these DOC values were not due to oil-type compounds.

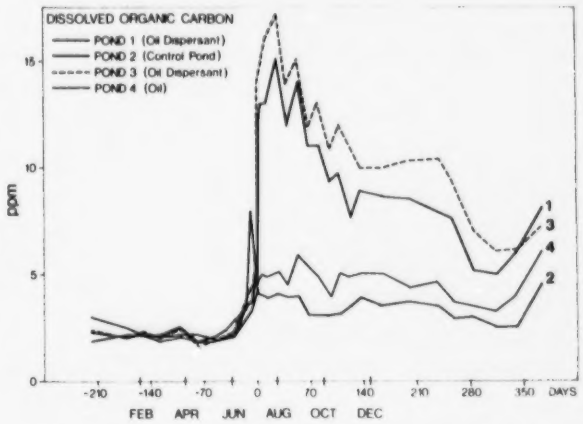



**Figure 8. Dissolved organic carbon concentrations.**

Unfiltered phosphorus, filtered phosphorus, ammonia, nitrate ion, total Kjeldahl nitrogen and sulphate ion concentrations are plotted in Figures 9, 10, 11, 12, 13 and 14, respectively. In some instances, inspection suggests there is an inverse correlation between nutrient concentrations (e.g., the unfiltered phosphorus and ammonia) and the phytoplankton biomass. The ammonia concentration in the ponds was sufficiently high to provide the nitrogen nutrient of the phytoplankton without requiring the uptake of nitrate ion (21) and its subsequent conversion.

All of the ponds had higher nitrate concentrations in the spring of 1978 and 1979. Maximum values occurred in March of both years, a time when the snow and ice cover over the ponds was beginning to melt. Although the ice in the centre of the ponds was still thick at these times, it was beginning to detach from the liners, permitting runoff water to enter the water column. A comparison of the water chemistry parameters of the snow and one of the ponds is given in Table 5 for February and March of 1978. Concentrations of ammonia, nitrate, TKN, and all of the phosphorus parameters are higher for the snow than for the pond water. The major ions ($Mg^{++}$, $Ca^{++}$ and $Cl^{-}$), DOC, DRS (dissolved reactive silica), $SO_4^{-2}$ and alkalinity values are lower for the snow than for the water column. Of all the water chemistry parameters analyzed for March and April, only the nitrate ion was accumulated in the water column. All of the forms of phosphorus and ammonia were undoubtedly utilized by the biota in preparation for the usual spring blooms of phytoplankton (22). In the early spring of 1979, extremely high concentrations of nitrate were found in the oil and control ponds, and these concentrations were twice as high as those determined for both oil-dispersant-treated ponds. Ammonia, which is the form of nitrogen generally utilized by the phytoplankton, was comparable in all of the ponds. The anaerobic conditions in

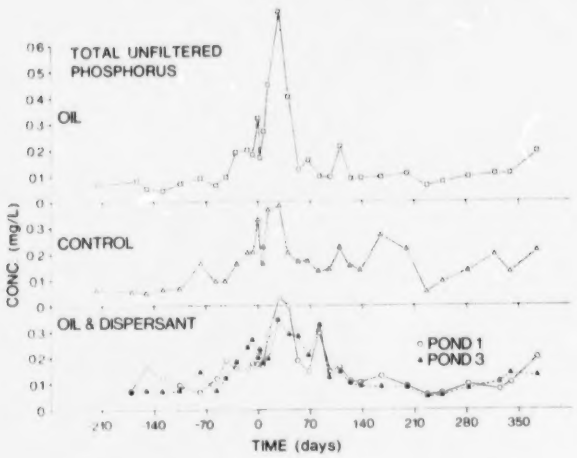



Figure 9. Unfiltered phosphorus concentrations.

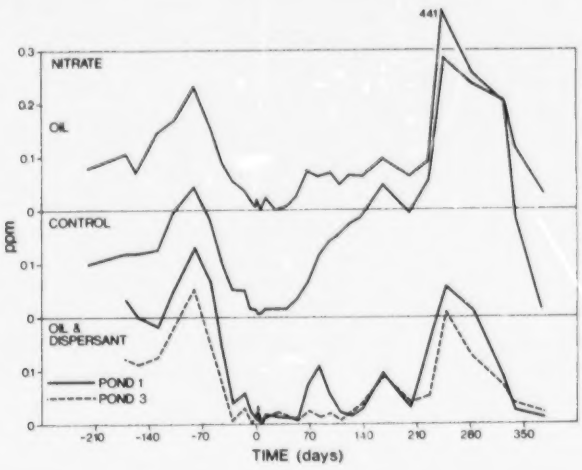



Figure 12. Nitrate ion concentrations.

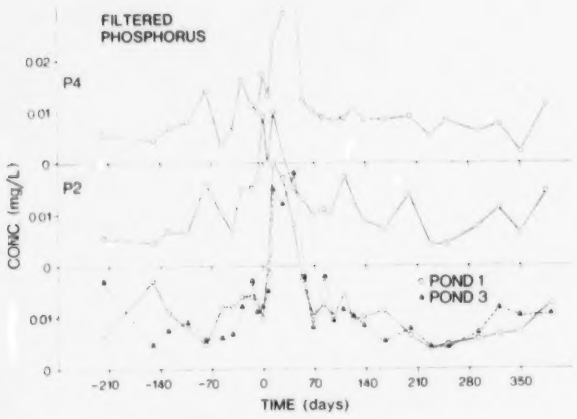



Figure 10. Filtered phosphorus concentrations.

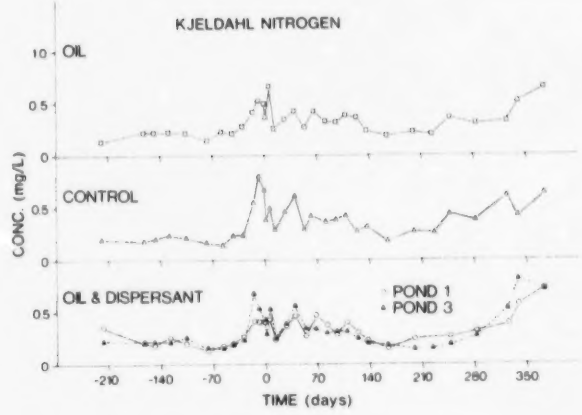



Figure 13. Kjeldahl nitrogen concentrations.

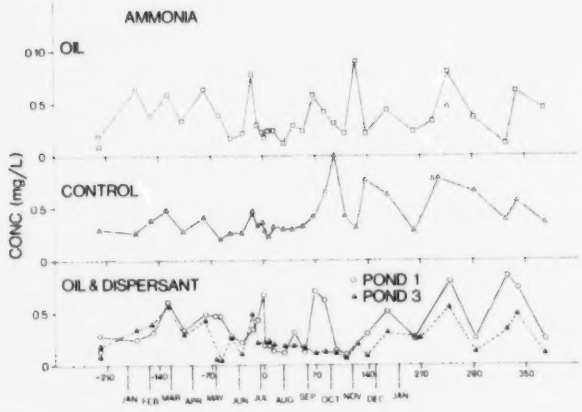



Figure 11. Ammonia concentrations.

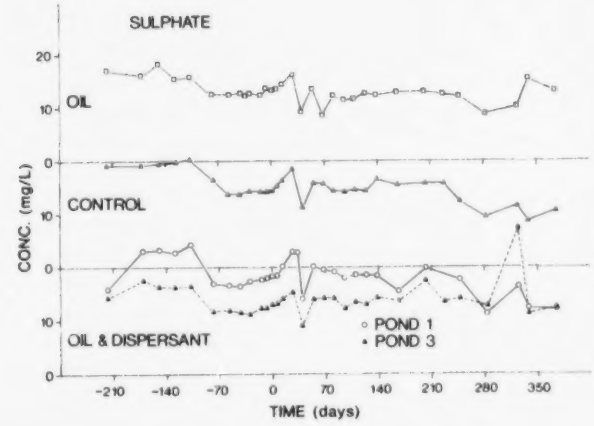



Figure 14. Sulphate ion concentrations.

Table 5. Comparison of Chemical Parameters Found in Pond Waters and in the Surrounding Snow (mg/L)

| Date | Parameter | $NH_3$ | $NO_3^-$ | TKN* | SRP† | Unfiltered P | Filtered P | DOC‡ | $SO_4^{-2}$ | DRS§ | Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 78-02-28 | Pond No. 2 | 0.047 | 0.128 | 0.237 | 0.0021 | 0.0075 | 0.0062 | 2.0 | 19.5 | 0.093 | 25 |
| | Snow | 0.120 | 1.02 | 0.258 | 0.010 | 0.014 | 0.014 | 1.1 | 2.5 | 0.024 | 1.0 |
| 78-03-22 | Pond No. 2 | 0.047 | 0.199 | 0.217 | 0.0016 | 0.0066 | 0.0063 | 2.2 | 20.5 | 0.068 | 24 |
| | Snow | 0.101 | 0.395 | 0.670 | 0.0106 | 0.040 | 0.031 | 1.7 | 2.5 | 0.01o | 0.1 |

* Denotes total Kjeldahl nitrogen.
† Denotes soluble reactive phosphorus.
‡ Denotes dissolved organic carbon.
§ Denotes dissolved reactive silica.

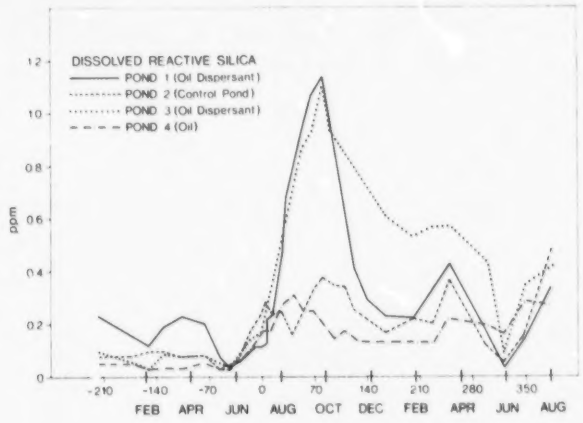



Figure 15. Dissolved reactive silica concentrations.

ponds Nos. 1 and 3 undoubtedly encouraged the presence of anaerobic growth which utilized the nitrate in both oil-dispersant ponds.

Dissolved reactive silica values are plotted in Figure 15. After treatment, both oil-dispersant ponds had high increases in this parameter. As silica is a vital component of diatoms, this component of the phytoplankton and periphyton was examined. Low numbers of diatoms in all of the ponds indicated that this parameter had no obvious effect. Subsequent laboratory studies (8) indicated that the silica had not been removed from the sediment but that it had probably been deposited from the atmosphere and retained in the water column of the dispersant-treated ponds by the dispersant.

# Protozoa, Zooplankton, Nekton and Zoobenthos

*by B.F. Scott, P.J. Wade, W.D. Taylor, D.B. Carlisle and N.B. Snow*

## METHODS

Protozoans were taken from the composite samples collected for phytoplankton, water chemistry and chlorophyll determinations. One litre of the composite was placed in a 1-L glass bottle containing 3 mL of modified Lugol's solution and stored at 4°C in a dark room. Prior to analysis, the sample volume was reduced by indirect filtration through a 10-μm mesh screen (23). Magnifications of x 400 and x 1000 were used for enumeration and identification. Identification of protozoa from preserved samples is difficult because the characteristics used to distinguish taxa are not visible. The identification initially was carried out using an internally consistent coding system. Many of the protozoa were later identified to the genera level and several to the species level (24,25).

During periods of ice cover, zooplankton were collected using a 2-L clear Van Dorn bottle to sample immediately below the ice surface, at mid-depth and just above the sediment. The contents of the Van Dorn samples were passed through a 40-μm mesh sieve, and the material on the sieve was combined and placed in a 200-mL storage bottle. Then 5 mL of carbonated water was added and the jar was topped with sugared-formalin solution (26). During ice-free periods, a clear Plexiglas 15-L Schindler-Patalas trap with 40-μm mesh sidearm and cup was used to collect samples from the near surface, mid-depth and near bottom from both sides of the catwalk. After each sampling, zooplankton caught in the sleeve were washed into the cup, which was then emptied into a large Plexiglas beaker. After the six samples were added, the volume was adjusted to 2 L and vigorously stirred. A 100-mL syringe was used to remove five representative aliquots which were passed through a 40-μm sieve, and the contents on the sieve were preserved as stated above. The contents remaining in the beaker were gently returned to the pond.

For analysis, the sample volumes were reduced to 25 mL by filtration through a 10-μm mesh screen. Enumeration and measurement for biomass estimates of all zooplankton in each sample were performed using a Wild M40 microscope at magnifications of x 25, x 40 and x 60 as well as at x 100 for some of the species. Identification was facilitated by using standard taxonomic texts (27,28,29,30,31).

Qualitative zoobenthos collections were made on a monthly basis during periods when there was no ice cover. A cleaned standard 48-oz can was fastened to the end of a long pole, and the open end of the can was gently scraped over the bottom sediment to collect the top surface of the sediment for a distance of about 20 cm. The contents of the tin were placed in a 1-L storage bottle, and the surface water was decanted after sufficient settling had occurred. If there was too much turbidity, the bottle was returned to the laboratories before decanting. The decanted water was replaced with the sugared-formalin solution. On the final sampling day, a number of samples were taken from each pond. In the laboratory, samples were sieved through a No. 35 Canadian Standard Sieve held underwater. Material remaining on the sieve was gently washed onto a white tray and then sorted. A Zeiss dissecting microscope was used to aid identification and then the organisms were preserved in 70% alcohol. Chironomid larvae were cleared, and the heads were removed and mounted for identification (32). Standard taxonomic references were used (33,34).

Occasionally, numbers of aquatic insects were estimated by inspection from a position directly over each pond. Representative samples of the insects were collected in a small net, preserved in 100% alcohol and identified.

## RESULTS

### *Zooplankton*

The logarithms of the zooplankton abundance are plotted in Figure 16. Zooplankton were abundant in all of the ponds prior to treatment. On the day following the treatment, the number of zooplankters in the oil-dispersant-treated ponds was reduced but not entirely eliminated. In these two ponds there was a definite difference between the upper 0.8 m of water and the lower part of the water column. The top layer was turbid and milky in appearance, and no zooplankters were visible in the samples. The bottom level of the water column was clear and there were many zooplankters. Since the samples from the three levels were combined, it appeared that the pond had a viable zooplankton population. One week after treatment, the ponds were uniformly turbid and only a few rotifers were collected in one of them. There was an almost

complete elimination of the crustacean zooplankton in these two ponds (ponds Nos. 1 and 3) until April of 1979, after the ice-cover had melted. After May of 1979, the zooplankton abundance in pond No. 1 increased to levels approximately equal to those in the control pond during June and July. Zooplankton populations in pond No. 3 were much lower than in pond No. 1 and the control pond at this time. The numbers in pond No. 3, however, were much higher than those recorded after treatment during the summer and fall of 1978. The zooplankton biomass is recorded in Figure 17.

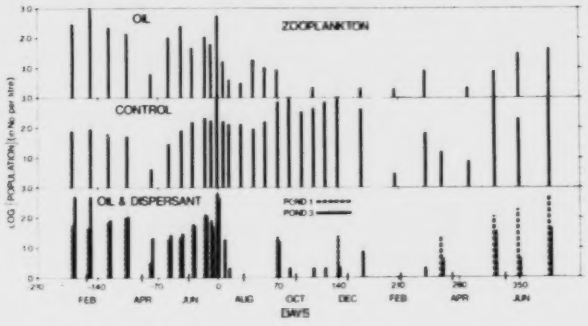



**Figure 16. Zooplankton populations in experimental ponds.**

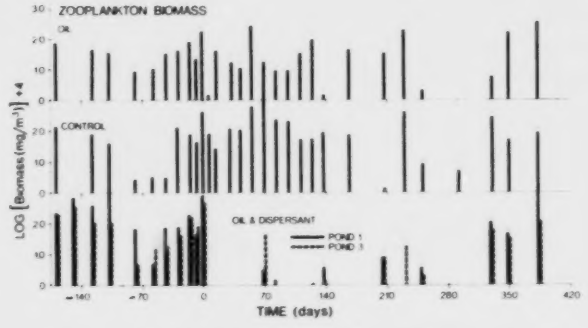



**Figure 17. Zooplankton biomasses in experimental ponds.**

Differences in the condition of some zooplankters were also noted. During September and October of 1978, an occasional *Chaoborus* sp. was collected. Those collected from the control pond exhibited normal rapid movement. In comparison, those collected from the oil-dispersant-treated ponds were lethargic, apparently near expiring. This might have been caused by an elimination of their normal food sources. These large Diptera, although there was less than one individual per 22.5 L of sample, would have contributed significantly to the zooplankton biomass and overwhelmed any contribution from smaller individuals. Therefore their contribution to the biomass was not included.

The zooplankton populations diminished in the oil-treated ponds, but there were usually one to seven individuals present in all of the samples until October of 1978. After April of 1979, the populations increased, indicating that the pond was recovering with respect to the zooplankton. This type of behaviour has been documented previously (6), when slightly greater amounts of oil were added under an ice-cover to a pond. At low concentrations, there was a reduction in the zooplankton populations, but the populations were not eliminated as found in the oil-dispersant-treated ponds. Major contributors to the zooplankton are given in Appendix D.

Since two methods were used to collect the zooplankton samples, two comparisons between the methods were conducted. The first was carried out in May of 1978, when both methods were used to collect composite samples from all of the ponds. The results are shown in Table 6a. There is agreement between the populations as well as the biomass estimates for the two methods of sampling. The major zooplankton species are common for both methods. The second comparison was made on the day of treatment in the auxiliary control pond (pond No. 5). Two locations on the water surface were chosen, and the water column was sampled just below the water surface and just above the sediment using both methods, permitting a sufficient time interval for the populations to re-establish themselves. These results listed in Table 6b show that despite the known patchiness of zooplankton (21), the two sampling methods give comparable results for this pond study. The biomass in all samples was dominated by *Polyarthra vulgaris* and *P. major*. Nauplii of Calanoida and Cyclopoida were significant in all of the samples as well. *Chaoborus* were found in all of the Schindler-Patalas trap samples but only in one Van Dorn sample. This observation can possibly be explained by the larger volume sampled by the Schindler-Patalas trap. The similarity of results obtained from both methods is extremely important, since samples taken by the Van Dorn sampler when there was an ice-cover could be compared with those collected by the Schindler-Patalas trap.

*Zoobenthos*

Qualitative zoobenthos results are listed in Table 7. The results for July 5, taken before treatment, indicate that dipteran larvae were present in all of the ponds. Twenty-seven days after treatment, pond No. 4 (the oil-treated pond) and the control pond still had a variety of Diptera, but the ponds treated with oil and dispersant had a reduction in the number of genera of Diptera present. This reduction was found in the oil-treated pond 28 days later on day 55. Samples from the control pond continued to have a number of zoobenthic species present, although

**Table 6a. Comparison of Zooplankton Sampling Methods for Composite Samples Taken May 23, 1978**

| | Pond No. 1 | | | | Pond No. 2 | | | | Pond No. 3 | | | | Pond No. 4 | | | | Pond No. 5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S-P trap* | | Sampler† | | S-P trap | | Sampler | | S-P trap | | Sampler | | S-P trap | | Sampler | | S-P trap | | Sampler | |
| Zooplankton | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| **Rotifera** | | | | | | | | | | | | | | | | | | | | |
| *Polyarthra vulgaris* | 11 | 3.61 | 23 | 3.61 | 31 | 4.28 | 31 | 2.52 | 52 | 7.07 | 144 | 13.0 | 207 | 27.0 | 129 | 10.3 | 12 | 0.83 | 4 | 0.48 |
| *P. major* | R | 0.25 | 1 | 0.36 | R | 0.08 | 3 | 0.83 | | | 3 | 0.87 | 6 | 1.9 | R | 0.1 | 3 | 1.06 | R | 0.26 |
| *Keratella cochlearis* | R | 0.01 | R | 0.01 | 13 | 0.38 | 2 | 0.60 | 3 | 0.10 | 2 | 0.07 | 4 | 0.3 | R | 0.03 | | | | |
| *K. crassa* | | | R | 0.01 | 1 | 0.05 | 3 | 0.13 | 1 | 0.05 | 3 | 0.11 | | | 1 | 0.04 | R | 0.01 | | |
| *K. quadrata* | | | R | 0.03 | 5 | 0.36 | 10 | 0.97 | R | 0.17 | | | | | | | R | 0.01 | | |
| *Synchaeta* sp. | R | 0.14 | | | | | | | | | | | R | 0.03 | | | R | 0.01 | | |
| *Lepadella patella* | 2 | 0.20 | R | 0.07 | 11 | 1.07 | 5 | 0.47 | 4 | 0.43 | 4 | 0.42 | 3 | 0.33 | R | 0.07 | 2 | 0.19 | R | 0.07 |
| *Trichocerca multicrinis* | | | | | | | | | R | 0.09 | | | | | | | | | | |
| *Asplanchna* sp. | R | 0.44 | | | | | | | | | | | | | | | | | | |
| **Crustacea** | | | | | | | | | | | | | | | | | | | | |
| *Chydorus sphaericus* | R | 1.22 | R | 1.36 | R | 3.66 | R | 5.08 | R | 2.96 | R | 4.23 | 2 | 9.09 | 2 | 17.6 | R | 4.12 | R | 2.40 |
| *Daphnia* sp. | 1 | 64.6 | 2 | 32.0 | R | 17.9 | | | | | | | R | 10.8 | R | 23.4 | 1 | 48.9 | R | 2.82 |
| *Scapholeberis kingi* | | | | | | | R | 2.07 | | | | | | | | | | | | |
| *Diacyclops bicuspidatus* | | | | | R | 1.39 | | | R | 0.57 | R | 0.56 | R | 0.54 | R | 0.94 | | | | |
| *Acanthocyclops vernis* | R | 0.25 | | | | | | | | | R | 1.79 | R | 0.89 | R | 1.18 | | | | |
| *Tropocyclops prasinus* | | | | | | | | | R | 0.49 | | | R | 0.47 | | | R | 0.15 | | |
| Copepodite | R | 0.70 | | | | | | | | | R | 0.94 | | | | | | | | |
| *Diaptomus minutus* | R | 0.19 | R | 3.50 | R | 0.83 | R | 0.47 | R | 0.19 | | | R | 0.17 | | | R | 0.60 | R | 0.46 |
| Nauplii | 6 | 0.40 | 7 | 0.98 | 11 | 1.08 | 7 | 1.51 | 32 | 5.06 | 62 | 21.0 | 24 | 1.69 | 12 | 3.05 | 17 | 1.36 | 3 | 0.50 |
| *Chaoborus* sp. | | | | | R | 0.24 | | | | | | | R | 0.25 | | | R | 0.25 | | |
| Total | 22 | 69.7 | 34 | 42.0 | 75 | 31.32 | 60 | 16.9 | 94 | 17.0 | 219 | 43.0 | 247 | 53.3 | 147 | 56.7 | 39 | 58.5 | 10 | 7.01 |

*Volumes used with Schindler-Patalas (S-P) trap for collection and analysis were 93.4 L and 23.3 L, respectively.
†Volumes used with Van Dorn sampler for collection and analysis were both 6.0 L.
A – Abundance (No./L).
B – Biomass X $10^4$ ($mg/m^3$).
R – Rare (less than 1/L).

this number diminished in November. No zoobenthos was found in any of the January 1979 samples. This might have resulted from scraping the same area under the ice-cover that was previously scraped for the sediment-oil analysis sample. The most exhaustive survey occurred in July of 1979, the last day of the experiment. These results, shown in Table 8, indicate that chironomid larvae had been re-established in the treated ponds. Indeed, in the interior treated ponds (ponds Nos. 3 and 4), the number of individuals collected was greater than in both control ponds.

During the sample collection on October 10, 1978, large larvae were noticed in ponds Nos. 1, 3 and 4. They were moving slowly near the water-sediment-air interface and made little effort to avoid collection. These were identified as *Pantala hymenea*, a migratory dragonfly (35) which usually emerges in August and returns to northern Minnesota. The gut was found to be empty when dissected. No larvae were found in pond No. 2.

### *Nekton and Surface Life*

For the purpose of this report, nekton will be defined as those entities that can take swimming motions in any direction in spite of turbulence. These animals, although not quantitatively sampled, were counted and collected for identification. Prior to treatment, water boatmen (Corixidae), back swimmers (*Notonecta* sp.) and frogs were visible in all of the ponds, and water striders (*Gerris* sp.) were observed on the surface of the ponds. Immediately after treatment, the water striders moved quickly away

**Table 6b. Comparison of Zooplankton Sampling Methods for Subsamples Taken July 5, 1978, from Pond No. 5**

| Zooplankton | Position No. 1 | | | | Position No. 2 | | | | Position No. 3 | | | | Position No. 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S-P trap* | | Sampler† | | S-P trap | | Sampler | | S-P trap | | Sampler | | S-P trap | | Sampler | |
| | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| **Rotifera** | | | | | | | | | | | | | | | | |
| *Polyarthra vulgaris* | 132 | 10.6 | 1669 | 134. | 475 | 38.0 | 478 | 38.2 | 128 | 10.2 | 56 | 4.44 | 2624 | 210. | 395 | 31.6 |
| *P. major* | 79 | 24.6 | 250 | 77.5 | 351 | 109. | 391 | 129. | 58 | 18.2 | 10 | 16.3 | 224 | 69.4 | 86 | 25.9 |
| *Keratella crassa* | R | | | | | | | | | | | | | | | |
| *Lecane* sp. | R | 0.02 | | | | | | | R | 0.02 | | | | | | |
| *Brachionus* sp. | | | | | | | | | | | | | | | R | 0.17 |
| **Crustacea** | | | | | | | | | | | | | | | | |
| *Chydorus sphaericus* | | | 2 | 2.66 | R | 0.12 | | | R | 0.12 | | | | | | |
| *Daphnia* sp. | R | 0.59 | | | R | 0.20 | R | 2.03 | R | 0.21 | R | 1.48 | R | 1.69 | | |
| *Bosmina longirostris* | | | | | R | 0.59 | | | | | | | | | | |
| *Alona* sp. | | | | | R | 0.41 | | | | | R | 0.34 | | | | |
| *Scapboleberis kingi* | | | | | R | 3.58 | | | R | 0.13 | 2 | 11.8 | R | 0.24 | | |
| *Diacyclops bicuspidatus* | | | 2 | 5.43 | R | 3.15 | | | R | 0.72 | 3 | 7.48 | 2 | 7.48 | 2 | 8.20 |
| *Eucyclops serrulatus* | | | R | 1.90 | R | 0.51 | | | | | | | R | 0.47 | | |
| *Tropocyclops prasinus* | R | 0.19 | | | | | | | R | 0.10 | | | | | | |
| *Diaptomus oregonensis* | R | 6.53 | 4 | 32.7 | 4 | 59.5 | 2 | 25.2 | 1 | 15.3 | R | 2.58 | 4 | 66.9 | 2 | 21.2 |
| Nauplii | 7 | 3.81 | 22 | 14.5 | 10 | 7.51 | 19 | 3.64 | 4 | 4.31 | 29 | 14.2 | 7 | 4.90 | 17 | 12.4 |
| *Chaoborus* sp. | R | 18.0 | | | R | 29.2 | R | 135 | R | 15.9 | | | R | 5.22 | | |
| Total | 219 | 48.0 | 1950 | 276 | 843 | 224 | 894 | 214 | 192 | 49.4 | 102 | 63.6 | 2862 | 291 | 503 | 101 |

*Volumes used with Schindler-Patalas (S-P) trap for collection and analysis were both 2.0 L.
†Volumes used with Van Dorn sampler for collection and analysis were both 6.0 L.
A – Abundance (No./L).
B – Biomass $\times 10^4$ ($mg/m^3$).
R – Rare (less than 1/L).

from the rapidly spreading oil-dispersant mixture, but slowly away from the oil front in pond No. 4. From the time of treatment until the following June, there were no water striders on the surface of any of the treated ponds. One week after treatment no nekton was observed in any of the treated ponds. Tadpoles were observed one week later in the control pond as well as in the oil-treated pond, but there were fewer individuals in the latter. At least one *Notonecta* was seen in the oil-treated pond two weeks later, and a salamander was observed in the control pond. No life was observed in the oil-dispersant-treated ponds. Four weeks after treatment, on August 1, tadpoles were still observed in ponds Nos. 2 and 4. There were many dead adult insects in the water column of the oil-treated pond. These insects probably emerged from the pupal stage but did not escape through the water-oil-air interface. On day 41, small frogs were observed in ponds Nos. 1 and 4 as well as either Corixidae or *Notonecta*, but these were fewer in number and smaller in size than observed in the control pond. A salamander was seen in the oil-treated pond.

The most intensive survey was conducted on August 23, 1978. At that time there were 39 water striders and a whirlygig (*Gyrinus* sp.) on the surface of the control pond, and 25 tadpoles and approximately 30 Corixidae or *Notonecta* in the water column. The oil-treated pond contained about five water boatmen or backswimmers, numerous tadpoles and a salamander, with about ten small frogs on the liner above the water surface. Both oil-dispersant ponds had fewer nekton than the other two ponds. By October 10, the temperature had decreased and no water striders were on the surface of the control pond, although there were either water boatmen or backswimmers in all of the ponds.

On April 24 of the following spring, the formerly turbid waters of the oil-dispersant ponds were clear. Water striders, water boatmen and backswimmers had appeared in all of the ponds. Salamanders were observed in pond No. 1.

**Table 7. Qualitative Zoobenthos Analysis of Individual Samples from Ponds Nos. 1 to 5 According to Sampling Date**

| Organism | 78-07-05 | | | | | 78-08-02 | | | | 78-08-29 | | | | 78-09-26 | | | | 78-11-21 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Phylum Mollusca | | | | | | | | | | | | | | | | | | | | | |
| *Physa* sp. | | | | | | | | | | | | | | | X | | | | | | |
| Phylum Cnidaria | | | | | | | | | | | | | | | | | | | | | |
| *Hydra* sp. | | | X• | X | | | | | | | | | | | X | | | | | | |
| Phylum Arthropoda | | | | | | | | | | | | | | | | | | | | | |
| *Hyalella azteca* | | | | | | | | | | | | | | | X | | | | | | |
| Subclass Ostracoda | | | | | | | X• | | | | X• | | | | X• | | X• | | | | |
| Order Ephemeroptera | | | | | | | | | | | | | | | | | | | | | |
| *Ephemera* sp. (naiad) | | | | | | | | | | | X | | | | X | | | | | | |
| *Caenis* sp. (naiad) | | | | | | | | | | X• | X• | | | | X | | | | | | |
| Order Trichoptera (larva) | | | | | | | | | | | | | | | X | | | | | | |
| Order Diptera (larvae) | | | | | | | | | | | | | | | | | | | | | |
| *Chrysops* sp. | | | | | | | | | X | | | | | X | | | | | | | |
| *Chaoborus* sp. | X• | X• | X• | X• | | | X• | | X• | X• | X• | X• | X• | | X• | | | | X• | X• | |
| *Procladius* sp. | | X | | | X | | X | | X | | | | | | X | X | | X | | X• | |
| *Chryptochironomus* sp. | X | X | X | X | | | | | | | | | | | | | | | | | |
| *Micropsectra* sp. | X | X | | X | X | | | | X | | | | | | | | | | | | |
| *Tanytarsus* sp. | X | X | | X | X | | | | X | | | | | | | | | | | | |
| *Pseudochironimus* sp. | | | | | | | | | | | | | | | X | | | | | | |
| *Endochironomus* sp. | | X | | | | | | | | | | | | | | | | | | | |
| *Cladotanytarsus* sp. | | | | | | | | | | | X | | | | | | | | | | |
| *Psectrotanypus* sp. | | X | | | | | | | | | | | | | | | | | | | |
| Unidentified Chironomids | X | | | | X | X | X | | X | | | | | | X | | | | | | |
| Chironomid pupae | | | | X | | | | | | | | | | | | | | | | | |

Note: Samples collected on January 23, 1979, contained no zoobenthos.
•Organisms contained in the zooplankton sample collected the same day.

**Table 8. Zoobenthos Abundance and Volumes Derived from Samples Taken July 24, 1979**

| Zoobenthos | Pond No. 1 (oil-dispersant)• | | Pond No. 2 (control) | | Pond No. 2 (control) | | Pond No. 3 (oil-dispersant upper level) | | Pond No. 3 (oil-dispersant lower level) | | Pond No. 4 (oil) | | Pond No. 5 (control) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. in sample | Vol.† | No. in sample | Vol. | No. in sample | Vol. | No. in sample | Vol. | No. in sample | Vol. | No. in sample | Vol. | No. in sample | Vol. |
| Arthropoda | | | | | | | | | | | | | | |
| Ostracoda | | | 1 | 5.45 | | | | | | | | | 1 | 71.0 |
| *Asellus* sp. | | | | | | | | | | | | | 12 | 1384 |
| Insecta | | | | | | | | | | | | | | |
| Zygoptera | | | | | 1 | 227 | | | | | | | | |
| Trichoptera | | | | | | | | | 1 | 1069 | | | | |
| Diptera (Chironomidea) | | | | | | | | | | | | | | |
| *Chryptochironomus* sp. | 1 | 234 | 1 | 4.27 | | | 21 | 13.4 | 23 | 78.5 | 8 | 40.2 | | |
| *Tanytarsus* sp. | 4 | 323 | 2 | 40.0 | | | 14 | 39.4 | 12 | 87.4 | 18 | 240 | 1 | 1060 |
| *Procladius* sp. | 2 | 1457 | | | | | 30 | 29.4 | 6 | 124.6 | 11 | 118 | | |
| Other chironomidae | 5 | 636 | 6 | 77.5 | 7 | 64.3 | 51 | 29.3 | 42 | 148.1 | 26 | 244 | 5 | 477 |
| Pupae | | | | | | | 1 | 9.0 | 4 | 103 | 3 | 160 | | |
| Oligochaeta | | | | | | | | | | | 1 | 1780 | 1 | 1507 |
| Mollusca | | | | | | | | | | | | | | |
| *Physa* sp. | | | | | | | | | | | 2 | 5775 | 1 | 3062 |
| Planorbidae | | | | | | | | | | | | | 3 | 5808 |

•Treatments are indicated in parentheses.
†Mean individual volume × $10^6$ ($\mu m^3$).

*Protozoa*

Protozoa are unicellular animals which consume algae, bacteria, fungi and other protozoa and which are grazed upon by zooplankton and zoobenthos. The total population and biomass of the protozoa in the water column for the ponds are shown in Figures 18 and 19, respectively. In a comparison of these figures, it is apparent that the protozoc in pond No. 4 were generally smaller than in the other ponds during the experiment. Immediately after treatment in the oil-dispersant-treated ponds, there was a reduction in the numbers of protozoa which only lasted four weeks. Their total numbers and biomass then returned to levels similar to those in the control pond. Inspection of the data at the genus or family level permits a better understanding of the effect of the treatment. Figure 20 shows the biomass of *Strobilidium* sp., and Figure 21 illustrates the biomass of *Halteria* plus *Askenasia*. These were often present in all of the ponds before treatment, but after treatment they were only found in the control pond. There were few *Halteria* and *Askenasia* in both oil-dispersant-treated ponds from the time of the addition of the chemicals until spring thaw the following year. *Strobilidium* was not observed in these ponds after treatment and only appeared in low numbers after the following spring. The oil-treated pond contained *Strobilidium* during October and November of 1978 but only occasional individuals of *Halteria* and *Askenasia* during this time. The *Askenasia* and *Halteria* had recovered by the following June. These two ciliates appear to be inhibited by the presence of oil or oil-dispersant mixtures, while *Strobilidium* is inhibited by the presence of oil and dispersant. *Halteria* usually feed on bacteria, whereas *Strobilidium* can also feed on phytoplankton, especially diatoms.

The *Vorticella* sp. biomass and zooflagellate biomass are shown in Figures 22 and 23, respectively. The bactivorous *Vorticella* was found in all of the ponds, including the control pond, after treatment. The zooflagellates were significant contributors to all of the ponds before and after treatment. These zooflagellates were not routinely identified to a lower taxonomic group. However, during the analysis of the phytoplankton, zooflagellates were frequently noted, and these were identified as *Monas*

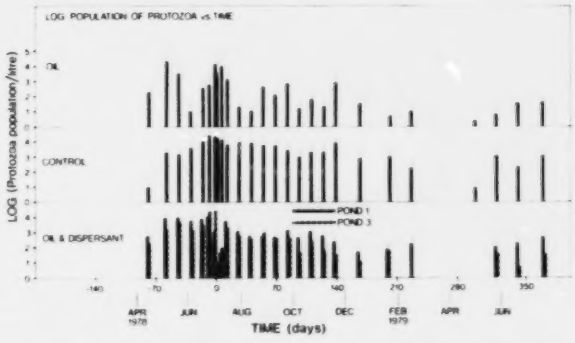



**Figure 18. Total protozoan populations in experimental ponds.**

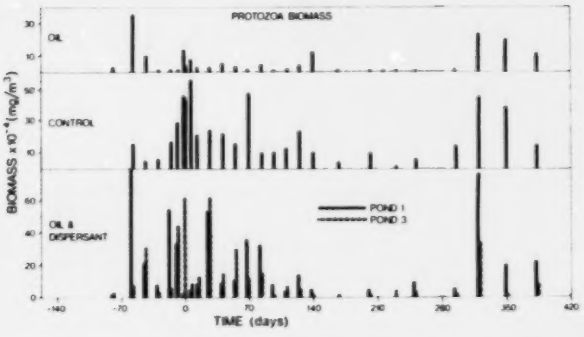



**Figure 19. Total protozoan biomasses in experimental ponds.**

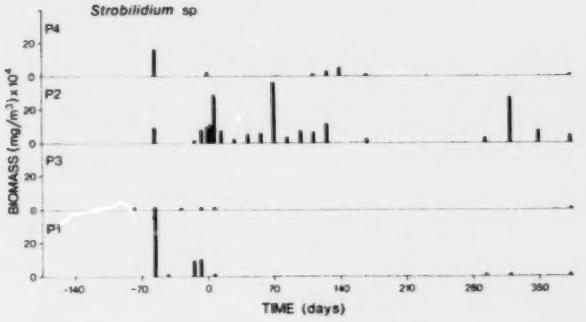



**Figure 20. Biomass of the ciliate *Strobilidium*.**

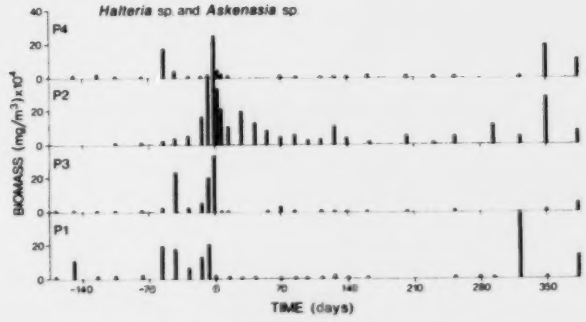



**Figure 21. Biomass of the ciliates *Halteria* and *Askenasia*.**

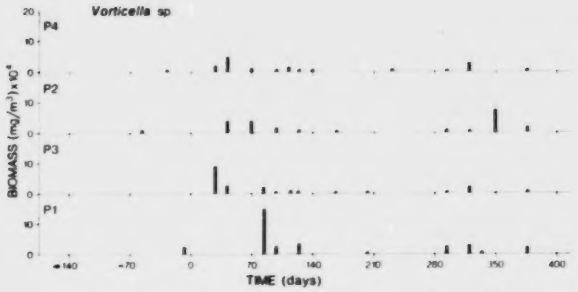



Figure 22. Biomass of *Vorticella*.

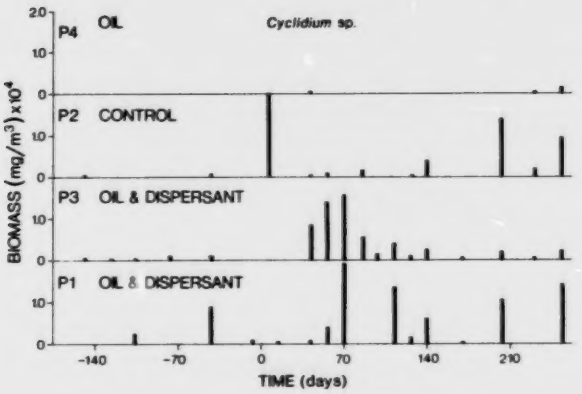



Figure 25. *Cyclidium* biomasses.

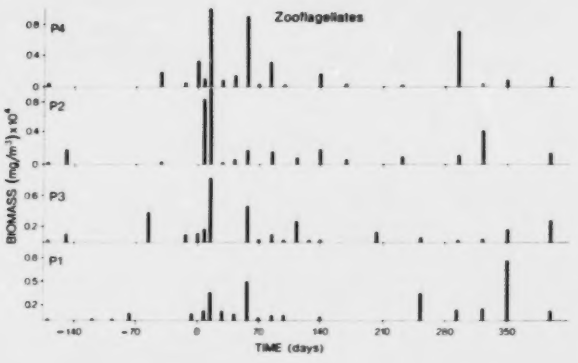



Figure 23. Zooflagellate biomasses.

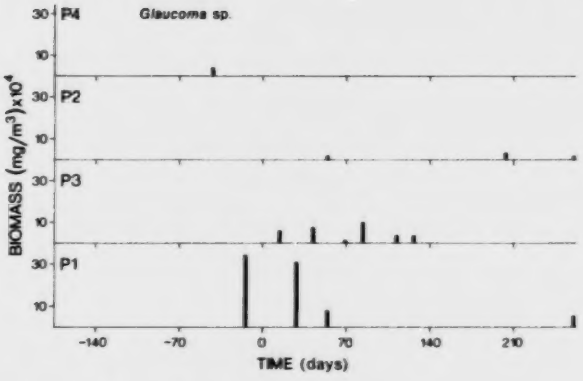



Figure 26. Biomasses of *Glaucoma*.

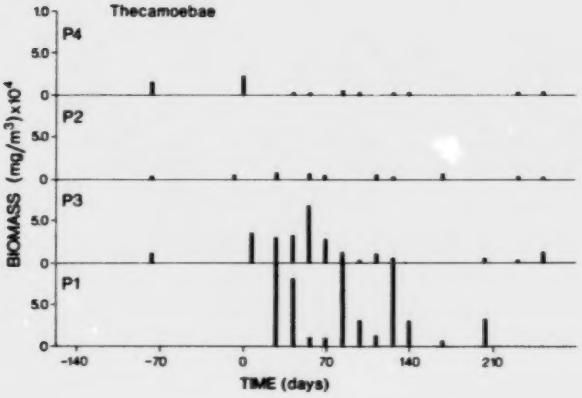



Figure 24. Thecamoebae biomass.

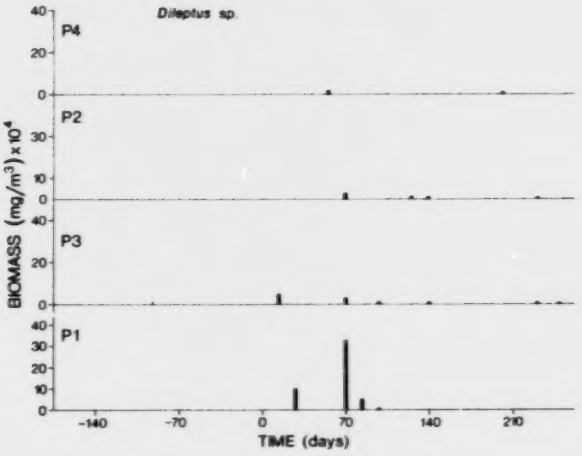



Figure 27. Biomasses of *Dileptus*.

sp. or *Desmarella* sp. with the occasional presence of *Salpingoeca* sp. (36). There were no conspicuous trends in their numbers or occurrence in the different ponds.

Figure 24 shows the biomass of thecamoebae, which are testaceous (shelled) amoeba that feed on filamentous algae. These protozoa were abundant in the oil-dispersant-treated ponds after the filamentous algae had become established. *Cyclidium* sp. biomass is illustrated in Figure 25 and that of *Glaucoma* sp. is shown in Figure 26. Both ciliates feed on bacteria and generally grow where there are high bacterial populations. *Cyclidium* was the most abundant in pond No. 3, the interior oil-dispersant-treated pond. The other oil-dispersant-treated pond also had higher biomass values for this protozoa than the control pond. Very few *Cyclidium* were found in the oil-treated pond. *Glaucoma*, also a bactivore, was found predominantly in the oil-dispersant-treated ponds. The biomass of *Dileptus*, a large ciliate which feeds on other protozoa, is illustrated in Figure 27. The largest biomass of this ciliate is found in the oil-dispersant-treated ponds.

At the time of spring thaw in 1979, the melting ice and snow contributed fresh water to all of the ponds. Before this time, *Cyclidium*, *Glaucoma*, thecamoebae and *Dileptus* had enhanced biomasses in the oil-dispersant-treated ponds. In these two ponds, *Halteria*, *Askenasia* and *Strobilidium* were inhibited, with the *Vorticella* and zooflagellates being unaffected by treatment. After the spring thaw of 1979, *Halteria*, *Askenasia*, *Chaenea*, *Strobilidium* and *Vorticella* were common and abundant in all of the ponds. During the summer, fall and winter of 1978, zooplankton grazing pressure in the treated ponds was greatly reduced. This may be the reason that slower moving protozoa, such as *Glaucoma*, could survive. *Halteria* and *Askenasia*, which usually dominate the protozoan biomass, are capable of quick, short movement and thereby have some defence against zooplankton predation.

# Bacteria

*by B.J. Dutka and A. Kwan*

## METHODS

Water samples were taken from the near surface, mid-depth and near bottom levels, and then refrigerated and returned to the laboratories for analysis. Aliquots from each sample were removed to determine a number of microbiological parameters.

The microbial biomass was estimated by the use of epi-fluorescence microscopic techniques (37). Heterotrophic bacterial counts were determined by using the spread plate procedure (37); one aliquot was incubated at 20°C for seven days and another at 4°C for 14 days. Sulphate reducing bacteria were estimated by Most Probable Number (MPN) procedures (37) as were sulphur oxidizing bacteria. Adenosine triphosphate (ATP) levels were determined by using a Dupont Biometer (38). The ATP data were recorded and reported as femtograms per litre.

Bacteria populations able to utilize nondegraded oil as the sole source of carbon were estimated by the use of spread plate techniques. Norman Wells crude oil was used. It was added to a carbon-free chemically defined solution (39) (10% v/v) and shaken for two months on a reciprocal shaker at 20°C. Foil was loosely placed over the top of the container to permit the more volatile components to evaporate. Bacterial populations that were able to utilize degraded oil as the sole carbon source were also determined by spread plate techniques. The carbon source was prepared by taking a fresh Norman Wells oil and carbon-free water mixture (10% v/v) and adding to it a Teflon membrane through which 100 mL of pond water had been filtered to provide the microbial inoculum. The mixture was shaken at 20°C for four months in the dark and then sterilized using a Toshiba 700 series microwave oven set at the maximum setting for 10 min. The oil-water-bacteria mixture and the oil-water mixture were refrigerated until required for incorporation into basal agar media for use in the spread plate procedures. The data, unless otherwise stated, are presented as the geometric mean derived from duplicate samples from the three water levels.

Toxicity tests on the dispersant were conducted using the bacterium *Spirillum volutans*, and these results are contained in Appendix E.

## RESULTS

The total microbial biomass in the ponds, as estimated by using the epi-fluorescence technique, is illustrated in Figure 28. Prior to treatment, the ponds had similar fluctuating populations, averaging $10^5$ bacteria per millilitre. Seven days after treatment, the oil-dispersant-treated ponds had the greatest microbial biomass, while the pond with only the oil had the least. The results for day 28 after treatment were similar; all of the ponds had a tenfold increase in the bacterial populations by late September (day 84) and then decreased to normal levels throughout the rest of the experiment. During this time, microbial populations in the control pond after treatment were generally lower than in the treated ponds.

Microbial biomass estimates expressed as femtograms ATP per 1000 mL of sample are displayed in Figure 29. The results show that the microbial biomass in the treated and untreated ponds fluctuated at about the $10^8$ femtogram level and that there was no consistent hierarchical pattern (ordering of response of systems with respect to treatment) dependent on the addition of oil or the oil-dispersant mixtures to the ponds.

These two methods estimate the total biomass, but do not show the effect of stresses on the various physiological groups that make up the microbiological community. Therefore a number of different types of plating and MPN procedures using various media were carried out to indicate changes within the community.

Geometric means of the recoveries from plating heterotrophic bacteria incubated at 20°C are presented in Figure 30. Following the spring thaw of 1978, high heterotroph populations were recorded. This was followed by a steady decrease in numbers until June when these populations reached densities that were similar to those normally observed in temperate climates. During this time there was no ordering of the results from the specific ponds.

Samples taken one week prior to and one week after treatment also did not exhibit any pattern dependent on treatment and remained at about $10^3$ to $10^4$ colonies per

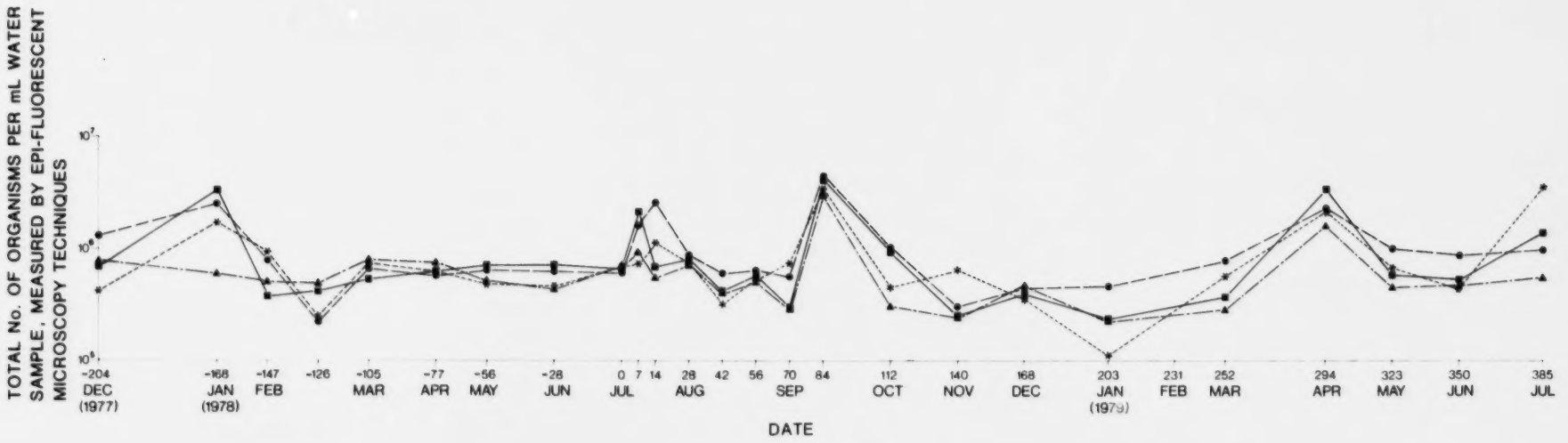



**Figure 28. Total microbial biomass determined using epi-fluorescence techniques.**

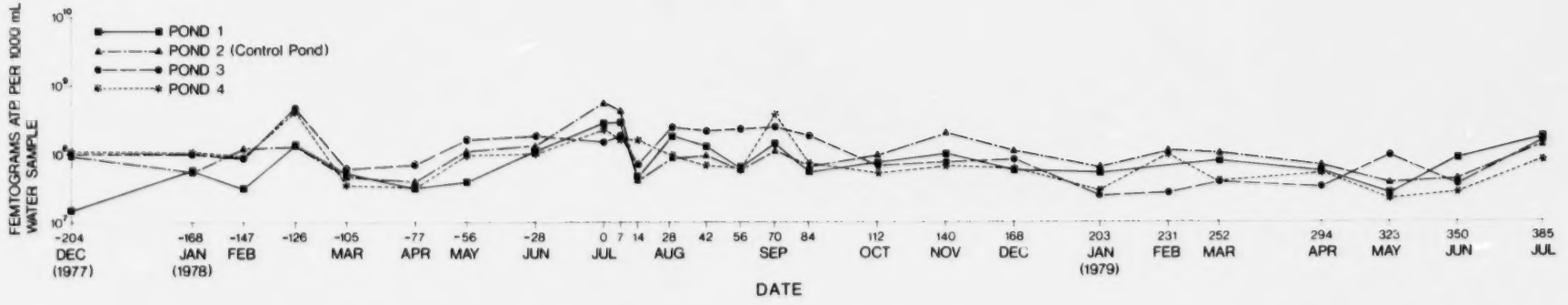



**Figure 29. Microbial biomass determined by ATP measurements.**

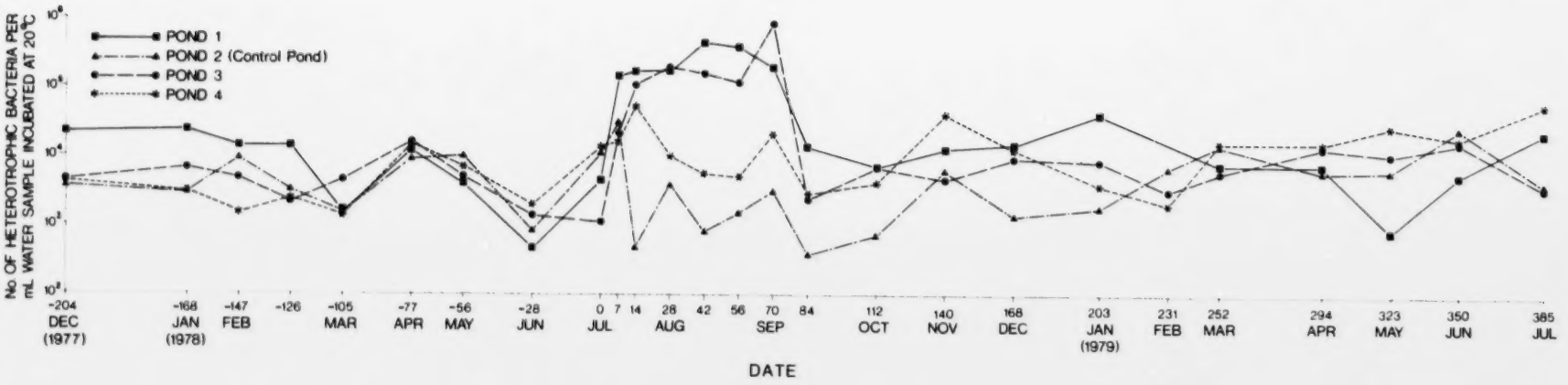



**Figure 30. Total heterotrophic bacterial populations in ponds (incubated at 20°C).**

millilitre. Two weeks after treatment, recoveries of colonies from the two oil-dispersant-treated ponds were a factor of 10 higher than those from the control pond, and the oil pond also exhibited densities higher than the control pond. This hierarchical pattern persisted until late September (day 84), with values recovered from the ponds treated with dispersant reaching counts of $10^6$ organisms per millilitre. This pattern continued but at lower values until January of 1979, after which time there were no clear patterns dependent on treatment and the counts fluctuated between $10^3$ and $10^4$ organisms per millilitre.

Heterotrophic population data are separated in Figure 31 to show the counts in the near surface and near bottom water samples over the study period. Prior to the June sample, the ponds exhibited a tendency for the heterotrophic populations from the bottoms of the ponds to be about equal to those collected from the near surface waters. All June samples and those from the control pond taken over the summer and fall had higher microbial densities in samples collected from the near bottom levels than from the near surface levels. In the oil-dispersant-treated ponds, there was a tendency for the top and bottom

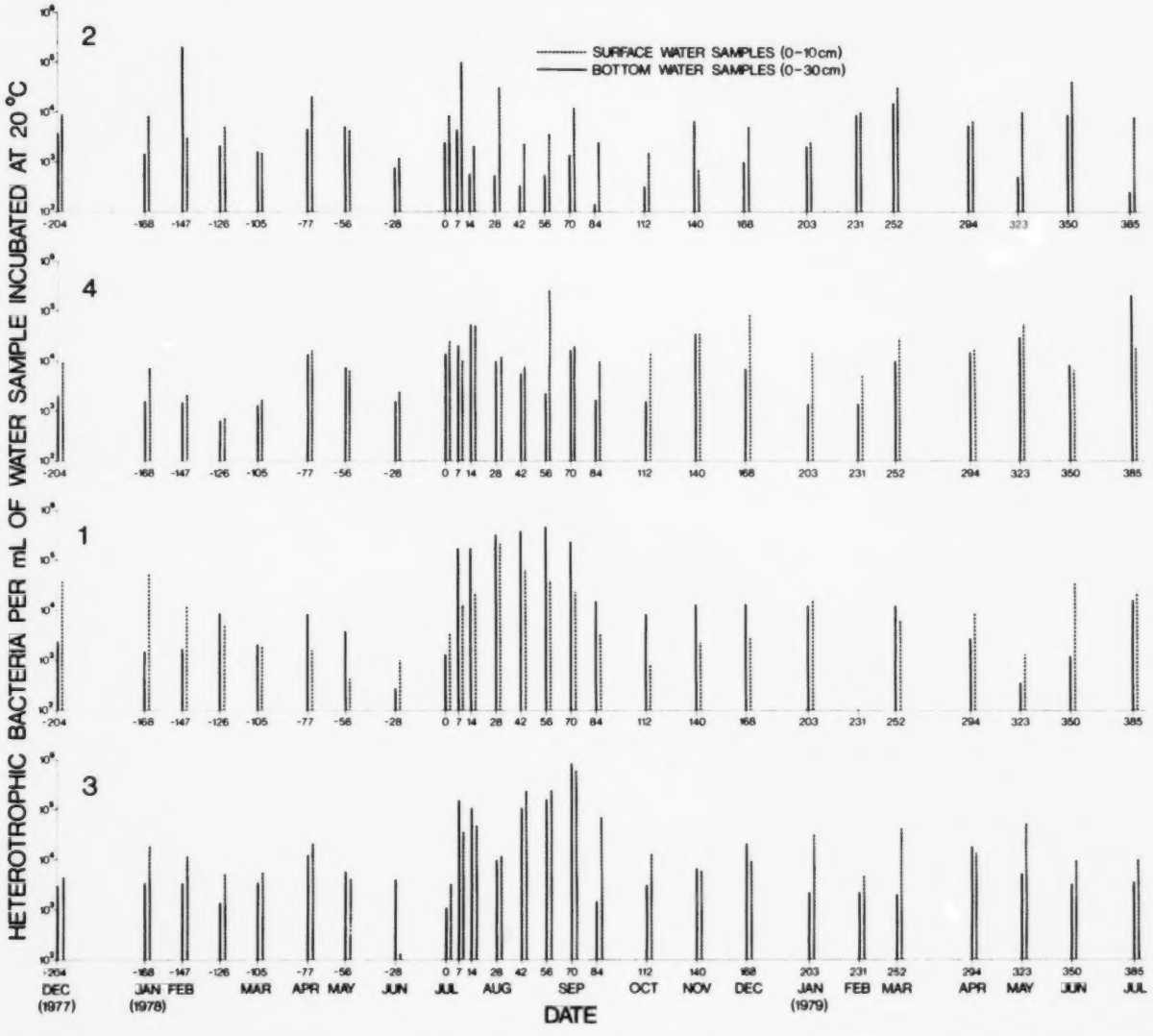

HETEROTROPHIC BACTERIA PER mL OF WATER SAMPLE INCUBATED AT 20 °C
SURFACE WATER SAMPLES (0-10cm)
BOTTOM WATER SAMPLES (0-30cm)
2
4
1
3
-204 -168 -147 -126 -105 -77 -56 -28 0 7 14 28 42 56 70 84 112 140 168 203 231 252 294 323 350 385
DEC (1977)
JAN FEB (1978)
MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
JAN FEB MAR APR MAY JUN JUL (1979)
DATE

**Figure 31. Heterotrophic bacterial populations in near surface (0-10 cm below surface) and near bottom (0-30 cm above sediment) water levels of experimental ponds.**

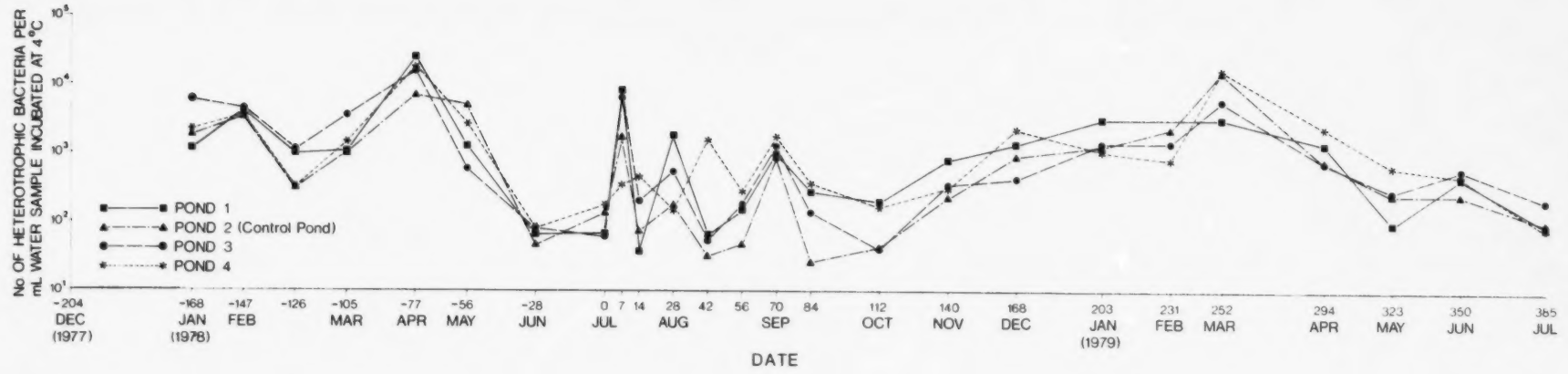



**Figure 32. Heterotrophic bacterial populations in ponds (incubated at 4°C).**

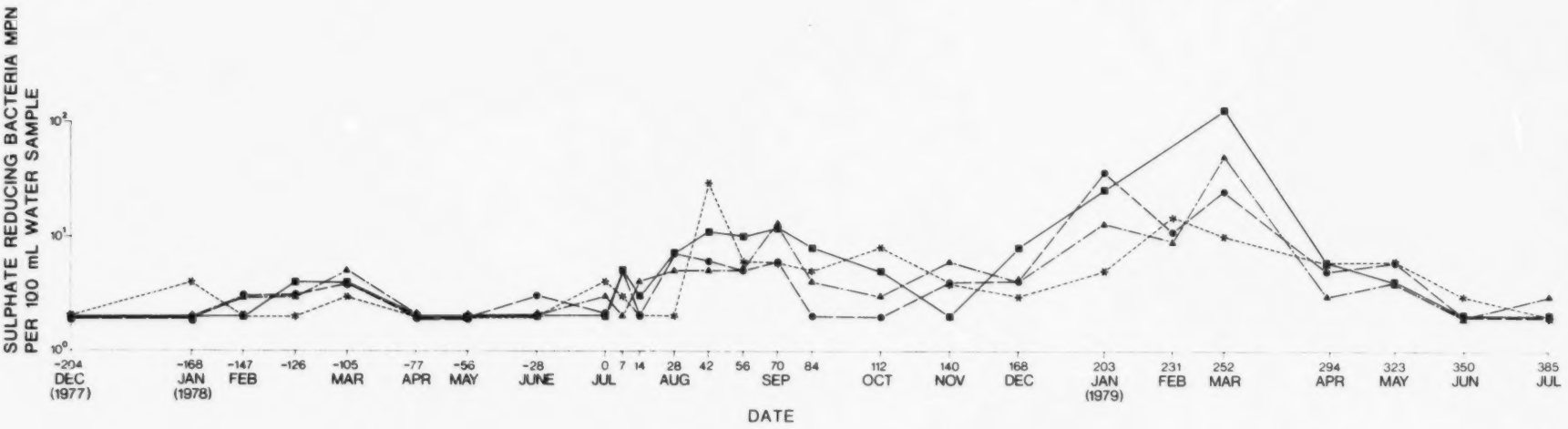



**Figure 33. Sulphate reducing bacterial populations in experimental ponds.**

populations to be about equal in pond No. 3 and for the near surface values to be higher than the near sediment populations in pond No. 1. The oil-treated pond exhibited trends similar to those of the control pond, but the heterotrophic densities were greater than in the control pond.

Recoveries of heterotrophic bacteria incubated at 4°C are plotted against time in Figure 32. These density estimates are significantly lower than those obtained by incubating at 20°C and show no ordering with respect to treatment before the chemicals were added. After treatment, the populations fluctuated widely, with the control pond usually having the lowest recoveries. Samples taken after day 84 indicated that these heterotrophic populations followed a similar pattern in all of the ponds, slowly peaking in March 1979 and then decreasing to their minimum levels in July.

There were low densities of sulphate reducing bacteria in all of the ponds, as illustrated in Figure 33. During the period from day 28 to day 56 after treatment, populations in the treated ponds were higher than in the control pond, with the highest populations recovered from the oil-treated pond. For the rest of the study no consistent hierarchical pattern was observed. Maximum populations occurred between January 1979 and March 1979 (day 203 to day 252), the period when there was an ice-cover over the ponds. After March the populations decreased. The recoveries of sulphate reducing bacteria from the near surface and near bottom water samples are plotted against time in Figure 34. When sulphur reducing bacterial populations were low, the densities from the near bottom samples were generally equal to the near surface. When there was an enhanced value, however, the near surface densities were usually greater.

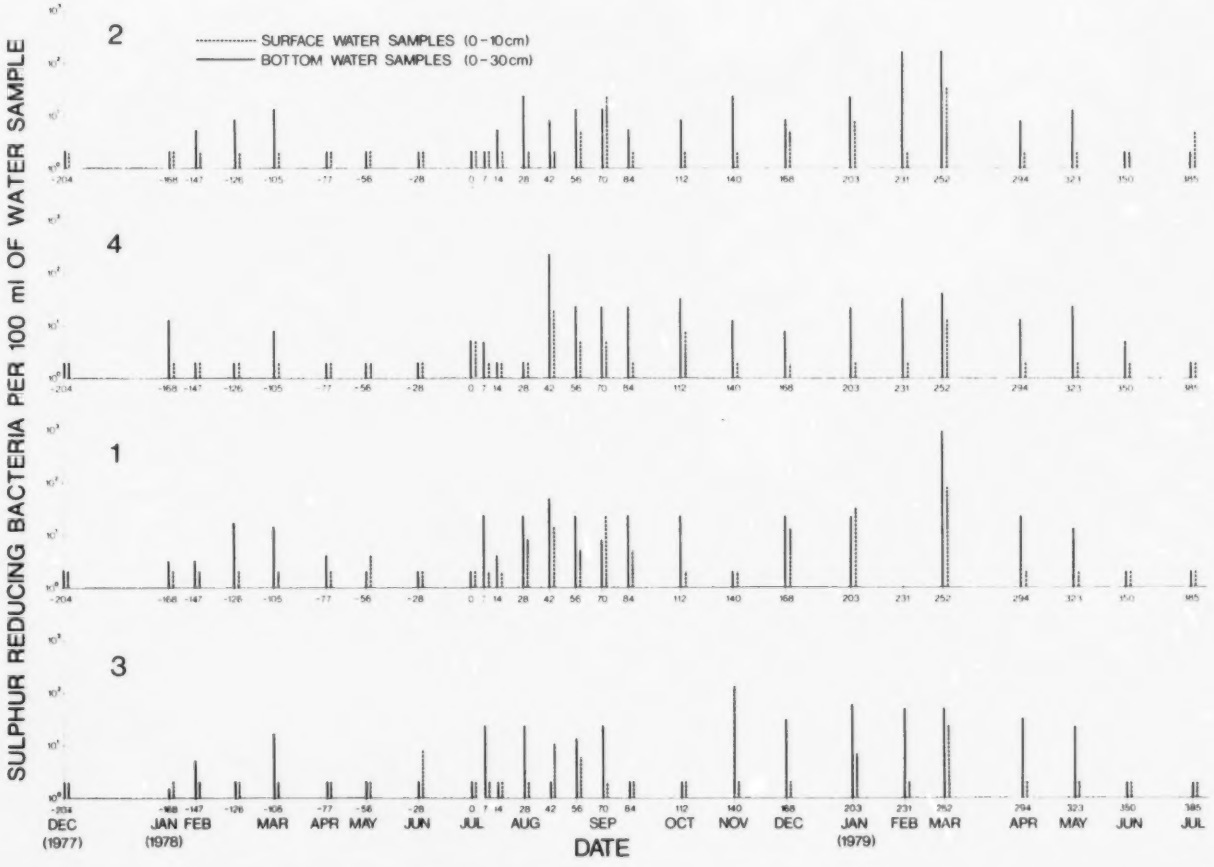



**Figure 34. Sulphate reducing bacterial densities in near surface (0-10 cm below water surface) and near sediment (0-30 cm above sediment) water levels in experimental ponds.**

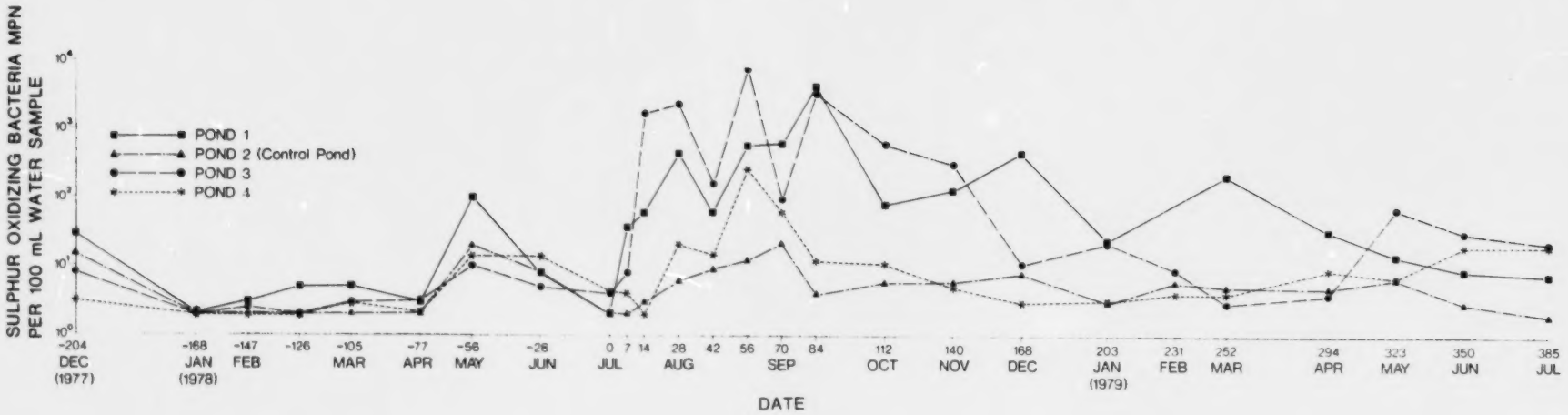



**Figure 35. Total sulphur oxidizing bacterial populations in experimental ponds.**

Sulphur oxidizing bacteria densities are plotted in Figure 35. Before treatment all of the ponds had similar low densities of this physiological group – less than 100 per millilitre. After treatment there was a rapid population increase of about two orders of magnitude in both oil-dispersant-treated ponds and a lower, but significant, increase in the oil-treated pond. Throughout the rest of the study, ponds Nos. 1 and 3, the oil-dispersant-treated ponds, usually maintained the highest populations of sulphur oxidizing bacteria. By the end of the experiment, the number of bacteria recovered from these two ponds approached the values determined on the first sampling day (day -204). Throughout the time from treatment to day 385, the control pond had the lowest populations. The populations recovered from the near surface and near bottom water samples are plotted in Figure 36. The populations recovered from the near bottom samples of the control pond are reasonably consistent, exhibiting three maxima which decline to minimum values in the summers after the two spring maxima. The oil-treated pond also exhibited three maxima, with the last one occurring for the bottom water samples in the summer of 1979 and the maxima having higher populations than those in the control after treatment. Population trends in the near bottom water samples from the oil-dispersant-treated ponds were not consistent between ponds Nos. 1 and 3, but both exhibited a number of maxima and minima, all at levels higher than those of the control pond.

The results of the plating experiments in which nondegraded and degraded oil were used as the sole carbon source are plotted in Figures 37 and 38. Before the addition of chemicals to the ponds, the number of bacteria able to grow on and utilize both types of oil was similar in all four

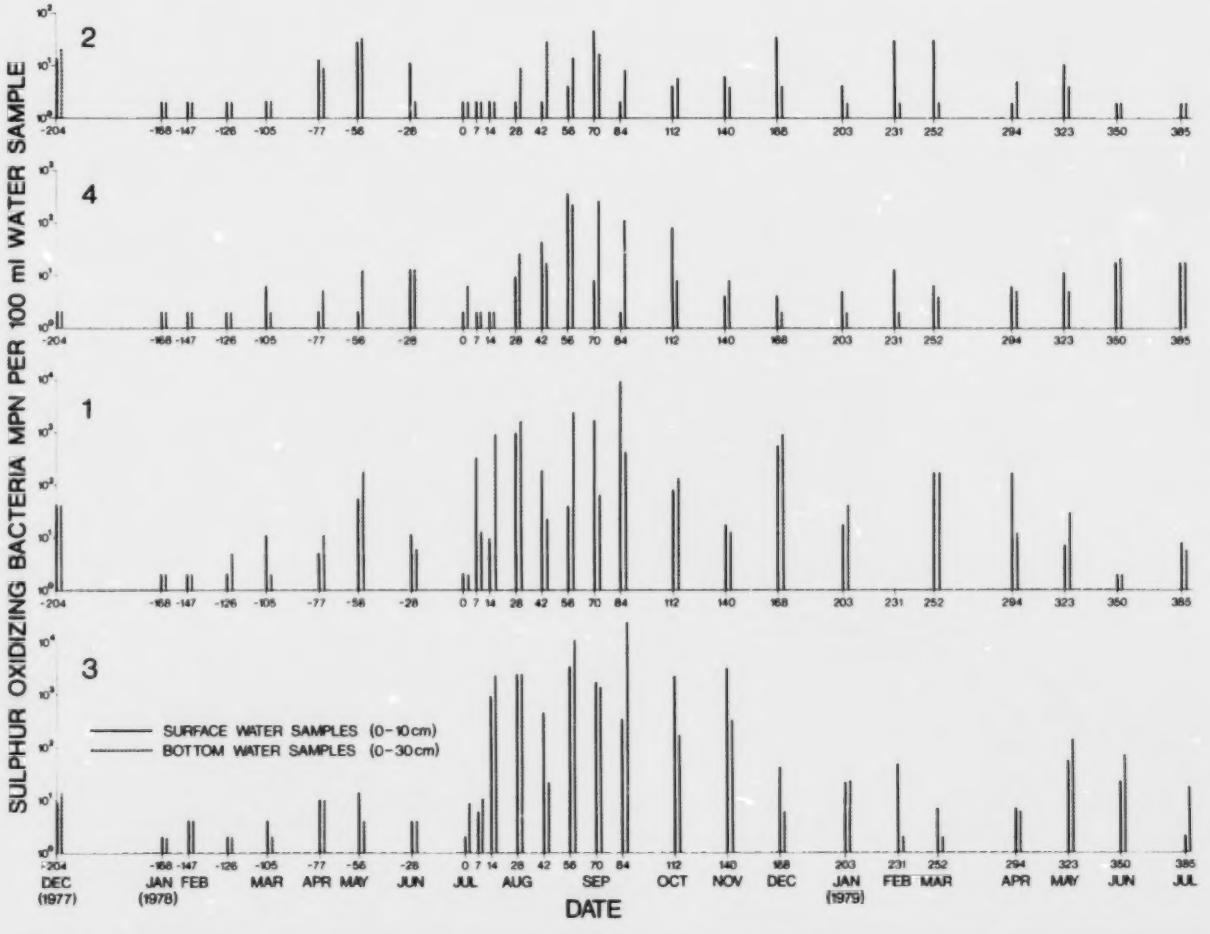



**Figure 36. Sulphur oxidizing bacterial populations in near surface (0–10 cm below surface) and near sediment (0–30 cm above sediment) water levels in experimental ponds.**

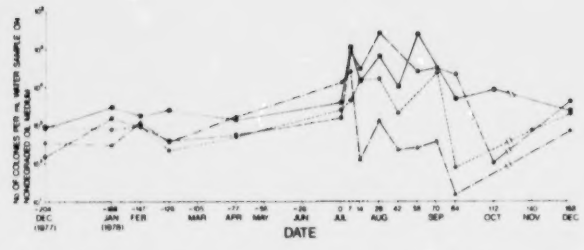



Figure 37. Bacterial recoveries from water samples where nondegraded Norman Wells crude oil is the sole carbon source.

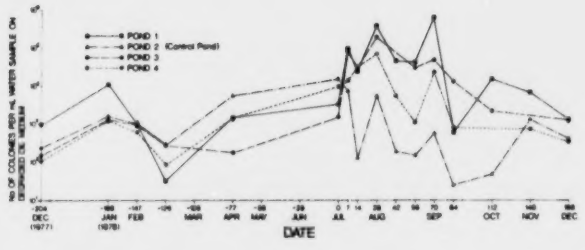



Figure 38. Bacterial recoveries from pond water samples where degraded Norman Wells crude oil is the sole carbon source.

ponds. The addition of oil and oil plus dispersant to the ponds results in a 10 to 100-fold increase in these microbial populations relative to the control pond, with the greatest increase found in the ponds treated with oil plus dispersant. The microbial populations shown in Figures 37 and 38 are similar in concentration and seasonal distribution patterns to those of the heterotrophs incubated at 20°C.

These microbial data show that although there is a minimal distortion of the total bacterial populations when oil and oil-dispersant mixtures are added to the water column, these added chemicals do cause a considerable shift in the composition of the bacterial community. This is particularly emphasized in the heterotrophic bacteria populations and the sulphur oxidizing bacteria. Lowering the incubation temperature for heterotrophs results in lower numbers of these bacteria being recovered, reflecting the influence of lower temperatures in the ponds themselves. Recoveries from the platings in which the oil, both degraded and nondegraded, was used as the carbon source were similar to the densities determined for the heterotrophic bacteria populations plated at 20°C. Since the three sets of results were similar with respect to population and seasonal distribution, this suggests that these bacteria in the treated ponds were able to use the oil as a carbon source. When the populations from the near surface and near bottom were considered, the ponds treated with oil and dispersant usually had more heterotrophs, sulphur oxidizing and reducing bacteria in the water column than the control and the oil-treated ponds.

# Mycology

*by James P. Sherry and Scott Kuchma*

## METHODS

Samples for mycological analysis were taken from the near surface, mid-depth and near bottom levels of the ponds and were immediately refrigerated and transported to the laboratories where they were processed within 30 h of collection.

The geo-aquatic fungi were enumerated using a membrane filtration procedure described in Dutka (37). Following triplicate filtration of appropriate aliquots, two of the membrane filters (0.45 $\mu$m) were implanted onto mARGPA agar (Appendix F) and were then incubated for five days at 15°C. The third membrane was plated onto mSTMEA agar and was also incubated at 15°C for five days. The mSTMEA is useful in the selective enumeration of pink and cream coloured yeast colonies.

Fungi capable of utilizing either nondegraded crude oil or degraded crude oil as the sole source of carbon were also enumerated. Appropriate volumes of pond water were filtered through 0.45-$\mu$m membrane filters which were then plated onto freshly prepared basal agar medium (medium OBA) containing either degraded or nondegraded crude oil (2 mL/22 mL medium); the control plates contained no oil. The inoculated plates were incubated for 21 days at 20°C and the fungal colonies were then enumerated as total colonies and were also judged as well-developed or strongly growing colonies. The subjective procedure was used to bias the data in favour of fungi that utilized the oil as a carbon source as opposed to fungi growing on background contaminants or impurities in the agar media. The oil media preparation is described in the methods section for bacteria. All fungal colonies were enumerated using a stereo microscope. All data are arithmetic mean values of triplicate or duplicate determinations and are expressed as colony-forming units (CFU) per 100 mL.

The water mould or aquatic phycomycete content of the ponds was monitored using two methods, one quantitative and the other qualitative. The number of viable water mould propagules in the water samples was determined using a spread plate technique that used 1-mL aliquots from each sample which were spread onto the surface of predried plates of PSP agar and then incubated at 15°C for 48 h. The number of typical phycomycete colonies growing on each plate was then enumerated, and all of the potentially positive colonies were transferred into pure culture onto PSP agar and stored at 5°C for subsequent identification. The qualitative method is referred to as the "baiting technique." A 50-mL water sample was placed in a sterilized battery jar; then 100-mL additions of 1% (w/v) NaCl solution, streptomycin (350 ppm) and sterile distilled water were made after which ten sterile, split hemp seeds were added to each jar. These baited samples were incubated at 20°C for three days. Then 650 mL of sterile distilled water was added and the incubation continued for an additional four days. At that time the number of colonized single seeds and clumps of colonized seed were enumerated as units. Individual colonized seeds and colonized seed clumps were transferred into pure culture on PSP agar and stored at 5°C for subsequent identification.

The isolates were identified on the basis of their asexual reproductive structures, which were induced using the following procedure. The isolates were grown on PSP agar at 15°C for 24 h; then six sterile hemp seeds were placed on the agar surface, concentric with the colony margin, and the incubation was continued for two more days at 15°C, after which time the hemp seeds were usually well colonized. The colonized hemp seeds were then transferred into glass petri dishes. Three seeds were placed in two dishes that contained distilled water and dilute salts solution (Table F-5, Appendix F), respectively. After 24 and 48 h, the developing fungal colonies around each seed were examined microscopically for characteristic asexual reproductive structures. Occasionally, it was necessary to repeat this procedure or to incubate the cultures overnight at 5°C and then let them stand at room temperature for 4 h to induce asexual sporulation. Potentially positive isolates were identified to the generic level before they were tabulated as confirmed isolates. Identifications were made using standard mycological keys and taxonomic references (40).

## RESULTS

*Geo-aquatic Fungi*

Figure 39 shows that before the oil and oil-dispersant applications, there was little apparent ordering in the individual ponds on the basis of the fungal data. However,

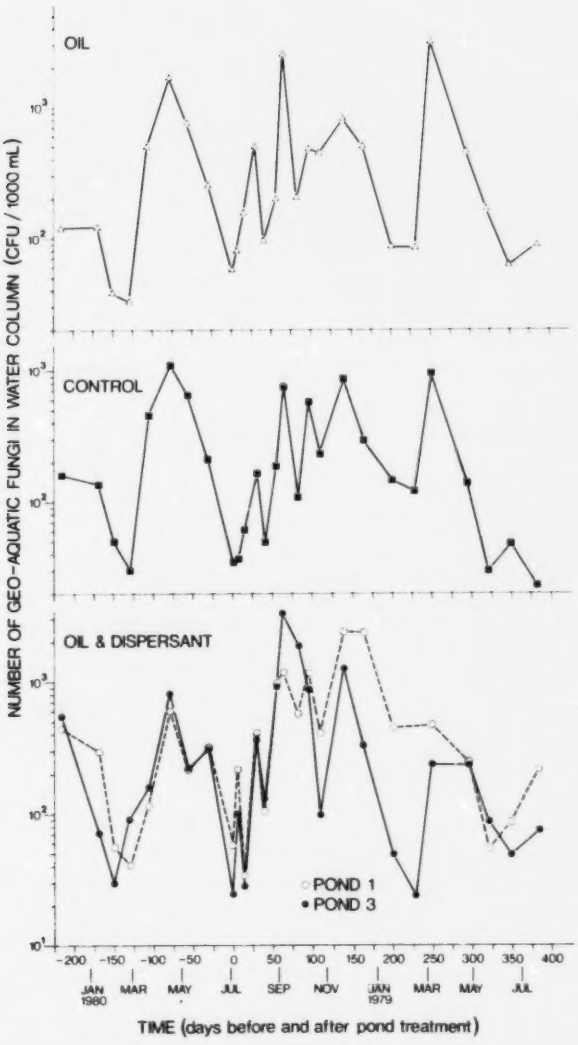



**Figure 39. Temporal distribution of viable geo-aquatic fungi in experimental ponds; data are presented as the geometric mean values of surface, mid-depth and bottom water samples.**

it can be seen from Table 9 that where the percentage of surveys in which the mean fungal levels exceeds the control pond levels, the numbers of fungi were generally higher in the treated ponds than in the control ponds. From the figure it is seen that the temporal distribution of the fungi in ponds Nos. 1 and 3 was very similar prior to treatment. Initially, the numbers of fungi in ponds Nos. 1 and 3 were less than in ponds Nos. 2 and 4, and during the pre-treatment period, the mean number of fungi in ponds Nos. 1 and 3 was less than in pond No. 2 by -768 CFU, or a par sampling differential of -86 CFU, as listed in Table 10. All of the ponds experienced a spring maximum in 1978, with the levels in ponds Nos. 2 and 4 being greater than in ponds Nos. 1 and 3.

**Table 9. Relationship between Fungal Levels in Treated and Control Ponds**

| Sampling period (days) | Percentage of samples in which the mean fungal levels exceeded control pond levels | | | |
|---|---|---|---|---|
| | Pond No. 1 | Pond No. 3 | Mean of ponds Nos. 1 and 3 | Pond No. 4 |
| -216 to 0 | 67 | 45 | 56 | 67 |
| 7 to 41 | 75 | 75 | 75 | 100 |
| 55 to 83 | 100 | 100 | 100 | 100 |
| 7 to 83 | 86 | 86 | 86 | 100 |
| 97 to 167 | 100 | 75 | 88 | 50 |
| 7 to 167 | 91 | 82 | 87 | 73 |
| 202 to 385 | 83 | 57 | 70 | 71 |
| 7 to 385 | 88 | 76 | 82 | 78 |

The addition of the oil and oil-dispersant mixtures to the ponds was followed by an immediate increase in the numbers of fungi in each of the treated ponds. This was the most obvious in ponds Nos. 1 and 3, the oil-dispersant-treated ponds in which the increase was from 42 CFU to 159 CFU. The number in the control pond only increased slightly during this time. By 14 days, however, a marked decrease had occurred in both oil-dispersant-treated ponds.

**Table 10. Comparison between the Numbers of Geo-aquatic Fungi in the Control and Treated Ponds**

| Sampling period (days) | Difference between the numbers of geo-aquatic fungi in the control and treated ponds (CFU) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (No. 1 - No. 2) | (No. 3 - No. 2) | Mean | (No. 4 - No. 2) | (No. 1 - No. 2)/n | (No. 3 - No. 2)/n | Mean | (No. 4 - No. 2)/n |
| -216 to 0 | -627 | -908 | -768 | 813 | -70 | -101 | -86 | 90.3 |
| 7 to 41 | 452 | 316 | 384 | 491 | 113 | 99 | 106 | 123 |
| 55 to 83 | 1675 | 4994 | 3335 | 1982 | 558 | 1664 | 1111 | 660 |
| 7 to 83 | 2127 | 5310 | 3719 | 2473 | 304 | 758 | 532 | 353 |
| 97 to 167 | 4377 | 548 | 2463 | 349 | 1094 | 137 | 616 | 87 |
| 7 to 167 | 6504 | 5858 | 6181 | 2822 | 591 | 533 | 562 | 257 |
| 202 to 385 | 220 | -673 | -227 | 2591 | 37 | -96 | -30 | 370 |
| 7 to 385 | 6724 | 5185 | 5955 | 5413 | 396 | 288 | 342 | 301 |

n – The number of sampling dates in interval.

This decrease may be related to the toxicity of the Corexit 9527, which at 25 ppm causes a reduction in the growth of pond water fungi (41), or to some other parameter. At this same time, the fungal levels in the other two ponds had increased. By day 28, the fungal levels in ponds Nos. 2 and 3 had increased to 385 CFU/100 mL and in pond No. 4 the increase was to 500 CFU/100 mL, while a much smaller increase had occurred in the control pond. By the next sampling the levels in all ponds had decreased, but the treated ponds had higher values than the control.

Samples collected between day 55 and day 83 after treatment showed that an increase had occurred in all of the ponds, but the increase was higher in the treated ponds. During the interval from 97 to 167 days, another increase in the fungal populations in all of the ponds was observed. All of the samples collected from pond No. 1 had higher fungal contents than those of the control pond, whereas only 75% of the sample sets from pond No. 3 exceeded the geo-aquatic fungal content of the control pond, as can be seen from Table 9. The mean difference per sampling day between the oil-dispersant-treated ponds and the control pond was less than during the 55- to 83-day period. For the first time since pond treatment, the mean difference between the oil-treated pond and the control pond was less than in the pre-treatment period.

The latter part of the study lasted from day 202 to 385. For the first time since pond treatment, the oil-dispersant-treated ponds contained fewer geo-aquatic fungi than the control pond. The numbers of geo-aquatic fungi in the oil-treated pond were once again higher than in the control pond, an observation that was heavily influenced by the results obtained on day 251. Only 72% of the oil pond sample sets between days 202 and 385 had higher fungal contents than the control pond as compared with 100% during the interval from 7 to 83 days after treatment.

The vertical distribution of the geo-aquatic fungi expressed as a percentage of the total number of samples is presented in Table 11. It is evident from the data that the maximum numbers of fungi occurred most frequently in the surface water samples from each pond and less frequently in the mid-depth samples. This observation is consistent with the fact that the surface microlayer, which contains a higher population of fungi and bacteria than the rest of the water column, is contained within the surface sample. Air deposition of fungal spores may have contributed to the higher fungal content of the surface samples. Conversely, Table 11 also shows that the minimum number of geo-aquatic fungi occurred least frequently in the surface samples and most frequently in the mid-depth and bottom samples.

The addition of oil and oil-dispersant mixtures to the ponds appears to have lessened the surface weighted distribution of geo-aquatic fungi in the water column. Prior to treatment, the maximum numbers of geo-aquatic fungi occurred most frequently in the surface water samples, especially in ponds Nos. 1, 3 and 4, while pond No. 2 had only a slightly higher Frequency of Occurrence of Maximum Numbers of geo-aquatic Fungi (FOMNF) in the surface water samples in comparison with the bottom samples. Pond treatment caused several short-term (7 to 83 days) changes in this distribution pattern. In the oil-dispersant-treated ponds, the FOMNF in the surface samples decreased by an average of 53% and the corresponding decrease in the oil treated pond was 24%. In contrast, there was an increase in the FOMNF in the surface waters of the control pond. At the same time, the FOMNF in the bottom water samples increased by an average of 25% in ponds Nos. 1 and 3 and decreased in ponds Nos. 2 and 4. Further changes occurred during the period from 97 to 167 days in which the FOMNF in the surface samples increased to 75% in ponds Nos. 1 and 3, the same frequency as in the control pond, whereas the FOMNF in the surface samples from pond No. 4 only increased slightly to 50%. At the same time the FOMNF in the bottom samples from pond No. 4 increased to 50%.

Over the period from 202 days to 385 days after treatment, the effects of the added chemicals were not

**Table 11. Effect of Pond Treatment on the Vertical Distribution of Geo-aquatic Fungi**

| | | Frequency of occurrence of maximum and minimum geo-aquatic fungal counts* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sampling | Pond No. 1 | | | Pond No. 2 | | | Pond No. 3 | | | Pond No. 4 | | |
| Count | period (days) | Surface | Mid-depth | Bottom | Surface | Mid-depth | Bottom | Surface | Mid-depth | Bottom | Surface | Mid-depth | Bottom |
| Maximum | -216 to 0 | 89 | 0 | 11 | 56 | 0 | 44 | 89 | 0 | 11 | 67 | 0 | 33 |
| | 7 to 83 | 43 | 28 | 29 | 86 | 0 | 14 | 29 | 28 | 43 | 43 | 28 | 29 |
| | 97 to 167 | 75 | 25 | 0 | 75 | 0 | 25 | 75 | 0 | 25 | 50 | 0 | 50 |
| | 7 to 167 | 55 | 27 | 18 | 82 | 0 | 18 | 45 | 19 | 36 | 45 | 19 | 36 |
| | 202 to 385 | 83 | 17 | 0 | 71 | 29 | 0 | 71 | 0 | 29 | 86 | 0 | 14 |
| | 7 to 385 | 65 | 23 | 12 | 78 | 11 | 11 | 56 | 11 | 33 | 61 | 11 | 28 |
| Minimum | Overall | 15 | 35 | 50 | 11 | 46 | 44 | 19 | 51 | 30 | 15 | 31 | 44 |

*Counts are expressed as a percentage of the total number of samples from each pond.

apparent on the FOMNF values in the surface water samples. Overall (day 5 to 385), the pond treatments decreased the FOMNF in the surface water samples by approximately the same amount. Ponds Nos. 3 and 4 both had higher FOMNF in the bottom water samples than the control pond.

The data obtained by culturing the pond samples on nondegraded and degraded oil are shown in Figures 40 and 41. These figures show that the oil and oil-dispersant additions had no obvious effects on the percentages of the viable geo-aquatic fungi capable of using either oil as a sole carbon source. Selective enumeration of strongly growing fungal colonies also failed to reveal any obvious treatment effects, as shown in Figures 42 and 43. Although minor differences did exist between numbers of oil-utilizing fungi in the ponds, they were neither substantial nor consistent enough to suggest the existence of a clear trend.

Because an increase in the number of oil degrading fungi in the treated ponds was not detected, the possibility exists that the enhancement of the geo-aquatic fungi numbers that occurred in the treated ponds may have resulted from a secondary effect. The toxicity of the added material would increase the amount of dead organic material in the ponds. A corresponding increase in the numbers of saprophytes, such as fungi, would then be expected. If the chemicals were also toxic to the components of the pond biota that also graze on the fungal hyphae or spores, then a general enhancement in the fungal numbers would be observed.

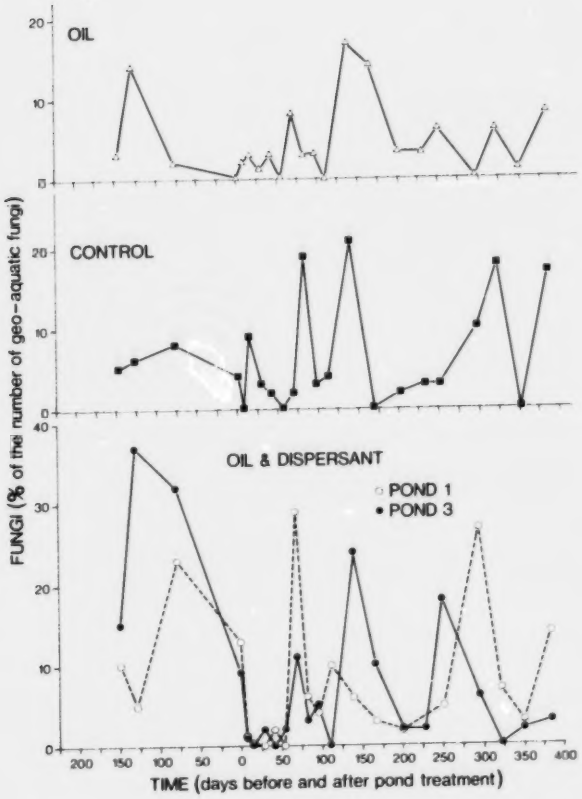



**Figure 40. Temporal distribution of fungi capable of growth on a medium containing Norman Wells crude oil as a sole source; data are presented as the geometric mean values of surface, mid-depth and bottom water samples and are expressed as a percentage of the numbers of viable geo-aquatic fungi in the respective samples.**

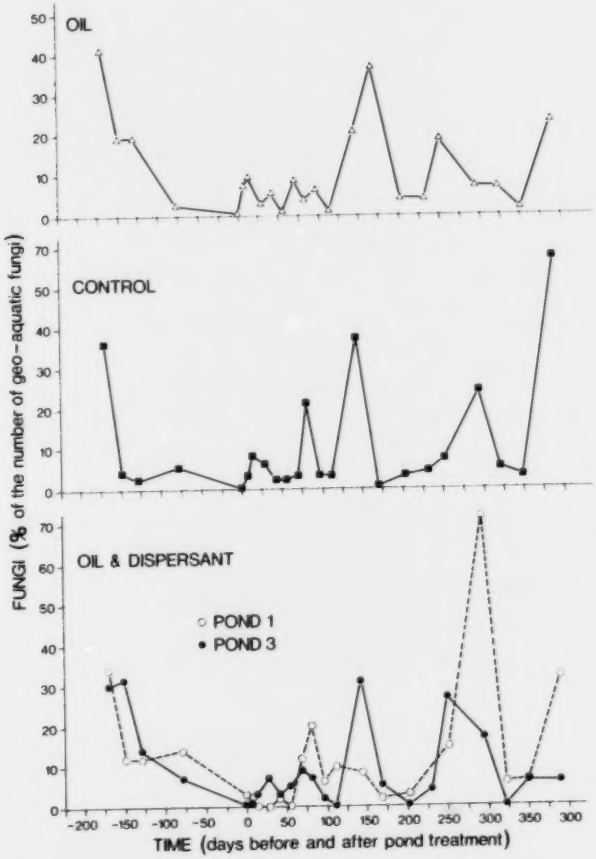



**Figure 41. Temporal distribution of fungi capable of growth on a medium containing biodegraded Norman Wells crude oil as a sole carbon source; data are calculated as geometric mean values of surface, mid-depth and bottom water samples and are expressed as a percentage of the numbers of viable geo-aquatic fungi in the respective samples.**

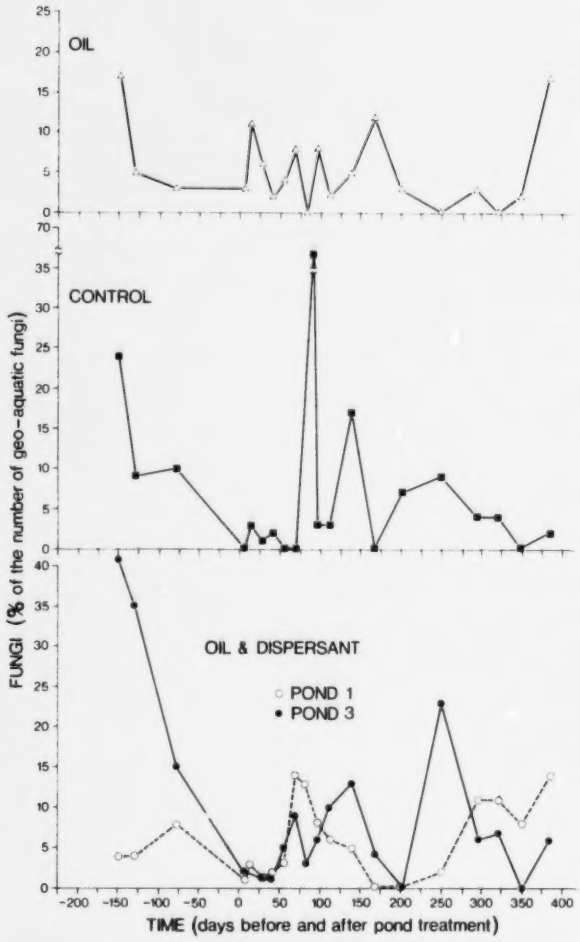



Figure 42. Temporal distribution of fungi capable of strong growth on a medium containing nondegraded Norman Wells crude oil as the sole carbon source; data are calculated as geometric mean values of surface, mid-depth and bottom water samples and are expressed as a percentage of the numbers of viable geo-aquatic fungi in the respective samples.

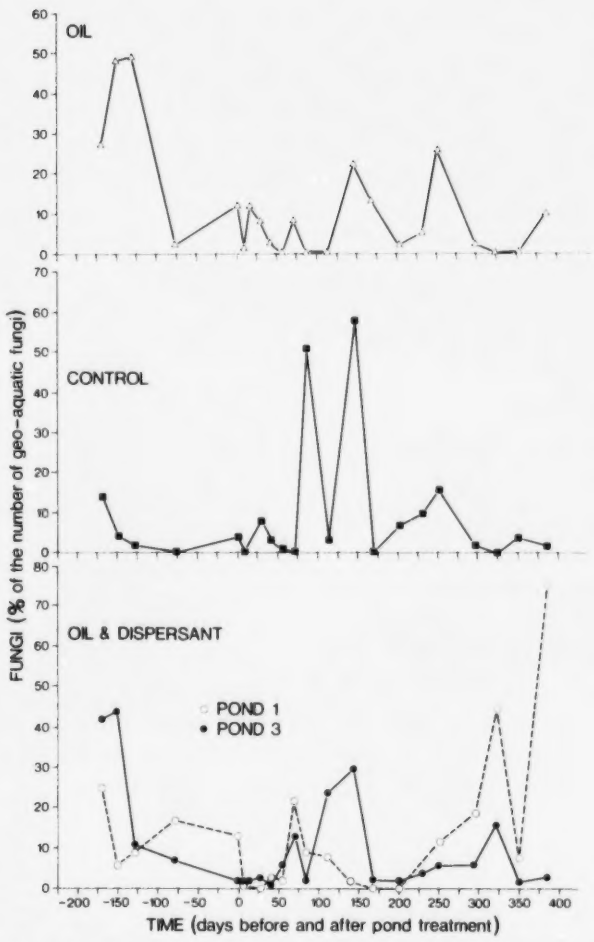



Figure 43. Temporal distribution of fungi capable of strong growth on a medium containing biodegraded Norman Wells crude oil as a sole carbon source; data are calculated as geometric mean values of surface, mid-depth and bottom water samples and are expressed as a percentage of the numbers of viable geo-aquatic fungi in the respective samples.

*Phycomycetes*

As can be seen from Table 12, the hemp seed baiting technique did not provide estimates of the phycomycete content of the pond water samples that were comparable with those obtained using the quantitative spread plate technique. The spread plate estimates, given in Table 13, suggest that differences existed in the phycomycete content of the ponds prior to treatment. In particular, the phycomycete content of the control pond was substantially less than that of the other ponds. Comparatively few phycomycetes were isolated from the ponds during periods of ice cover, suggesting that these fungi do not thrive under ice. Immediately after treatment (7 to 14 days), the phycomycete levels in the ponds changed. The control pond experienced a large increase to 19 CFU/50 mL, whereas smaller increases occurred in ponds Nos. 1 and 4, with pond No. 3 experiencing a decrease.

During the period of 28 to 111 days after treatment, the number of phycomycetes detected in the control pond decreased to 8.1 CFU/50 mL, whereas increases of 9.2, 27.2 and 5.3 CFU/50 mL occurred in ponds Nos. 1, 3 and 4, respectively. The difference between the phycomycete levels in the treated and control ponds was larger than during the pre-treatment stabilization period, indicating

**Table 12. Comparison between the Spread Plate Technique and the Hemp Seed Baiting Technique to Determine the Number of Phycomycetes in the Pond Water Samples (expressed as geometric mean for values from surface, mid-depth and bottom samples)**

| Technique | -216 | -168 | -149 | -128 | -106 | -77 | -66 | -29 | 0 | 7 | 14 | 28 | 41 | 55 | 69 | 83 | 97 | 111 | 139 | 157 | 202 | 230 | 251 | 296 | 322 | 350 | 385 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pond No. 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SPE* | | | 0 | 0 | 0 | 1 | 0 | 0 | 1.3 | 0 | 1.3 | 3.3 | 1.9 | 0 | 1.3 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1.3 | 1.3 | 1.4 | 1.6 |
| Hemp† | 1.3 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 7.2 | 0 | 1.3 | 3.1 | 3.3 | 5.3 | 1.6 | 1.4 | 2.6 | 2.2 | 1 | 1.6 | 0 | 0 | 0 | 2.6 | 2.6 | 2.0 | 1.4 |
| Pond No. 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SPE | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.3 | 1 | 1 | 0 | 0 | 0 | 0 | 2.4 | 0 | 0 | 0 | 0 | 0 | 0 | 1.3 | 0 | 1 | 0 |
| Hemp | 2 | 0 | 0 | 1.3 | 0 | 0 | 1.6 | 3.8 | 2.2 | 1.3 | 1.4 | 1.4 | 1 | 1.3 | 0 | 0 | 1.6 | 2.1 | 0 | 0 | 0 | 0 | 0 | 1.6 | 1 | 1 | 0 |
| Pond No. 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SPE | | | 0 | 0 | 0 | 1.6 | 2.3 | 1.4 | 0 | 0 | 1 | 6.7 | 1.4 | 1.4 | 1 | 1.9 | 0 | 2.5 | 0 | 0 | 0 | 0 | 0 | 1.8 | 1 | 1.3 | 1.3 |
| Hemp | 0 | 0 | 0 | 0 | 0 | 0 | 3.6 | 2.4 | 3.1 | 0 | 0 | 2.3 | 2.3 | 3.4 | 2.1 | 1 | 1.8 | 1.6 | 0 | 0 | 0 | 0 | 0 | 2.5 | 1.3 | 1.6 | 1 |
| Pond No. 4 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SPE | | | 0 | 0 | 0 | 1.3 | 0 | 1.4 | 0 | 1.4 | 0 | 1 | 0 | 1.8 | 1 | 2.3 | 0 | 1 | 1.3 | 0 | 0 | 0 | 0 | 1.4 | 1 | 1 | 1.3 |
| Hemp | 1.9 | 0 | 0 | 0 | 1.4 | 2.8 | 2.9 | 1.8 | 7.7 | 1 | 1.3 | 0 | 2.5 | 1.8 | 1 | 1 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 3.1 | 2.5 | 1 | 0 |

*SPE – Spread plate estimate (CFU/mL).
†Hemp – Hemp seeds colonized per 50 mL.

**Table 13. Spread Plate Estimate of the Numbers of Phycomycetes in Pond Water Samples**

| Pond number | Mean number of phycomycetes (CFU/50 mL) per sampling interval (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | −216 to 0 | 7 to 14 | 28 to 111 | Difference* | 296 to 385 | Difference | 28 to 385 | Difference |
| 1 | 5.5 | 10.8 | 20 | 11.9 | 23 | 13 | 15.7 | 9.8 |
| 2 | 0 | 19.2 | 8.1 | – | 10 | – | 5.9 | – |
| 3 | 12.6 | 8.2 | 35.5 | 27.4 | 23 | 13 | 21.1 | 15.2 |
| 4 | 6.4 | 11.7 | 17 | 8.9 | 20 | 10 | 13.6 | 7.7 |

*Difference between the number of phycomycetes in treated pond and the control pond for given sampling dates, which has then been averaged over the interval of interest.

**Table 14. Generic Identity of Pond Water Phycomycetes**

| Collection date | Pond number and genera isolated | | | |
|---|---|---|---|---|
| | Pond No. 1 | Pond No. 2 | Pond No. 3 | Pond No. 4 |
| −216 | *Saprolegnia*(1)* *Pythium*(3),*Aphanomyces*(1) | *Saprolegnia*(2) | | *Saprolegnia*(8) |
| −168 | | | | |
| −149 | | | | |
| −128 | | *Saprolegnia*(2) | | |
| −106 | | | | *Saprolegnia*(3) |
| −77 | *Saprolegnia*(1) | | *Saprolegnia*(3) *Achlya*(1) | *Saprolegnia*(11) |
| −66 | *Saprolegnia*(1) | *Saprolegnia*(3) *Pythium*(1) | *Saprolegnia*(23) | *Saprolegnia*(10) *Achlya*(1) |
| −29 | | *Saprolegnia*(10) | *Saprolegnia*(12) | *Saprolegnia*(7) |
| 0 | *Saprolegnia*(23) | *Saprolegnia*(10) | *Saprolegnia*(9) | *Saprolegnia*(23) |
| 7 | *Saprolegnia*(11) | | | *Saprolegnia*(8) |
| 14 | *Saprolegnia*(5) | *Saprolegnia*(5) | *Saprolegnia*(1) | *Saprolegnia*(2) |
| 28 | *Saprolegnia*(16) | *Saprolegnia*(5) | *Saprolegnia*(21) | *Saprolegnia*(1) |
| 41 | *Saprolegnia*(16) | *Saprolegnia*(1) | *Saprolegnia*(13) | *Saprolegnia*(8) |
| 55 | *Saprolegnia*(15) | *Saprolegnia*(2) | *Saprolegnia*(17) | *Saprolegnia*(7) |
| 69 | *Saprolegnia*(5) | | *Saprolegnia*(1) | *Saprolegnia*(2) |
| 83 | *Saprolegnia*(3) | | *Saprolegnia*(8) | *Saprolegnia*(9) |
| 97 | *Saprolegnia*(8) | *Saprolegnia*(17) | *Saprolegnia*(5) | *Saprolegnia*(5),*Pythium*(1) |
| 111 | *Saprolegnia*(8) | *Saprolegnia*(6) | *Saprolegnia*(14) | *Saprolegnia*(9) |
| 139 | *Saprolegnia*(2) | | | *Saprolegnia*(3),*Pythium*(2) |
| 157 | *Saprolegnia*(4) | | | |
| 202 | | | | *Saprolegnia*(6) |
| 296 | *Saprolegnia*(11) *Pythium*(1) | *Saprolegnia*(5) | *Saprolegnia*(14) *Pythium*(1) | *Saprolegnia*(15) |
| 322 | *Saprolegnia*(9),*Achlya*(3) | *Saprolegnia*(1) | *Saprolegnia*(2),*Pythium*(1) | *Saprolegnia*(9) |
| 350 | *Saprolegnia*(12) | *Saprolegnia*(3) | *Saprolegnia*(7) | *Saprolegnia*(2) |
| 385 | *Saprolegnia*(6),*Pythium*(1) | | *Saprolegnia*(3) | *Saprolegnia*(2) |

*Numbers in parentheses denote the total number of isolates from hemp seed baits and spread plates.

that stimulation of the phycomycete populations may have occurred. Phycomycete population levels in the treated ponds remained higher than in the control pond for the duration of the spring and early summer of 1979 (days 296 to 385).

The water moulds isolated using the hemp seed baiting techniques and the PSP spread plates were identified to the generic level, and those recovered are listed along with the corresponding isolation frequencies for each in Table 14. The vast majority of the isolates belonged to the genus *Saprolegnia*. In freshwater aquatic systems, *Saprolegnia* spp. are thought to function as fast-growing primary colonizers of dead organic matter. *Phythium* spp. and *Achlya* spp. were also occasionally isolated from the ponds, but only *Saprolegnia* was recovered from the control pond after treatment. Unfortunately, the data obtained from the ponds are too limited to conclude that this is an effect of the treatment.

# Discussion

The purpose of the experiment was to follow the fate of oil and oil-dispersant mixtures and to study their impact on the food web. To ascertain properly the impact of the chemicals on the biota, the concentrations as a function of time and the fate of the added material must be considered. In an earlier report (8), these aspects were described and it is useful to reiterate the findings here. Of particular importance is the concentration of the oil in the water column and a measure of the oil on the water surface and in the sediment. The measurable thicknesses of the oil slicks on the surface of the treated ponds are illustrated in Figure 44. Figures 45 and 46 show the concentrations of the oil and dispersant in the water column. Table 15 lists the amounts of oil found in the sediment from each pond. Surface slicks were initially quite thick, resulting from the undispersed oil and dispersed oil droplets returning to the surface. These slicks disappeared within 70 days. In the oil-treated pond there was an initial high pulse of oil in the water column which decreased to about 1 ppm after one day. In the oil-dispersant-treated ponds, there was an initial high pulse of oil in the water column as well as a simultaneous but lower pulse of dispersant. The oil concentrations in the water remained above 2 ppm for 55 days after treatment in pond No. 1 and for 84 days in pond No. 3. Appreciable amounts of oil were found in the sediment at day 55 after the addition of the chemicals in all treated ponds. The final distribution of the oil determined 385 days after treatment is shown in Figure 47. Most of the residual oil (53%) in the oil-treated pond was found in the sediment, with about 25% of the oil being unaccounted for. In the oil-dispersant-treated ponds, about 30% of the oil was found in the sediments with about 47% of the oil

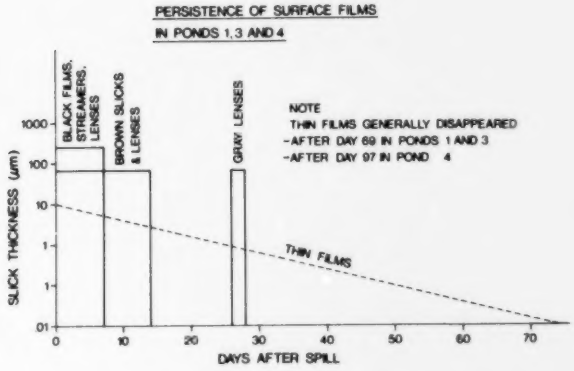



Figure 44. Surface oil thicknesses.

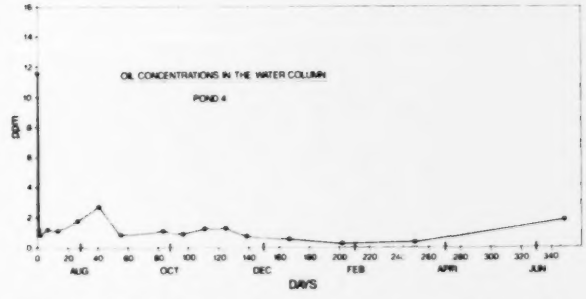
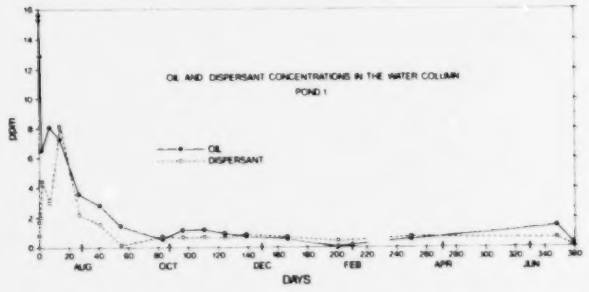



Figure 45. Concentration of oil in the water column of the oil-treated pond (No. 4).



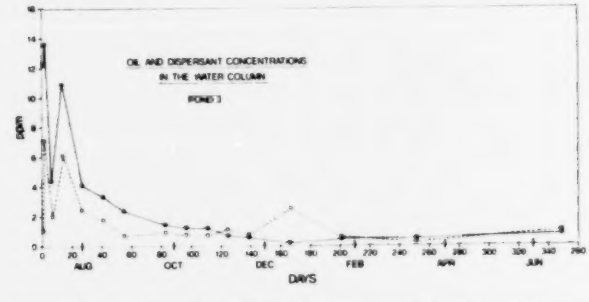




Figure 46. Concentration of oil and dispersant in the water columns of the oil- and dispersant-treated ponds (Nos. 1 and 3).

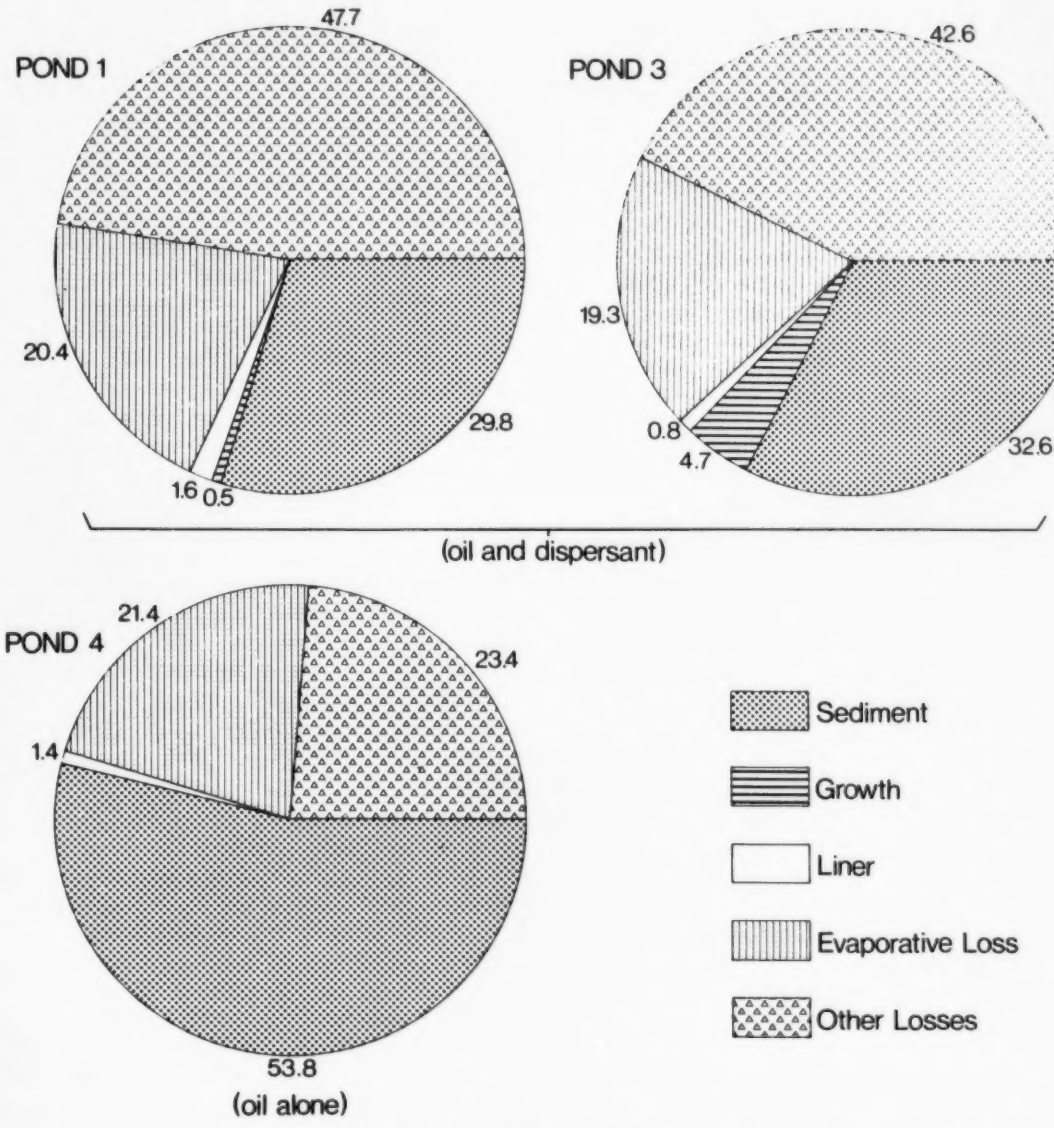



**Figure 47. Final distribution of the oil in all treated ponds (expressed as percent of added oil).**

being unaccounted for. Ponds Nos. 1 and 3, the two oil-dispersant-treated ponds, generally yielded similar results. However, they differed in detail, such as in the concentrations of oil in the water column and the amount of oil recovered from the attached algal growth. Slight differences in the biology of these two ponds reflect this variation. This section of the report will correlate changes in the biota with the fate of the oil and the interdependence within classes of the biota caused by the treatment.

Oil or oil-dispersant films on the water surface were inhospitable to surface insects such as *Gerris* sp. Although a surface sheen was not measurable after 70 days, the avoidance of the treated ponds by surface insects, despite an abundance of surface debris, indicates some residual effect of the added chemicals. After the spring of 1979, surface insect populations on all of the ponds indicated that the oil was not influencing the surface, although there was still oil in each treated pond.

**Table 15. Amounts of Oil Recovered from Sediment**

| Date | Elapsed time after treatment (days) | Pond No. 1 (oil+D)* (mg/g)† | Pond No. 2 (control) (mg/g) | Pond No. 3 (oil+D) (mg/g) | Pond No. 4 (oil) (mg/g) |
|---|---|---|---|---|---|
| 78-08-02 | 28 | 0.27 | 0.08 | 0.13 | 0.21 |
| 78-08-29 | 55 | 0.20 | 0.03 | 0.09 | 0.17 |
| 78-09-26 | 83 | 0.03 | 0.01 | 0.16 | 0.30 |
| 78-10-10 | 97 | 0.20 | 0.01 | 0.28 | 0.30 |
| 78-10-24 | 111 | 0.13 | 0.02 | 0.75 | 3.99 |
| 78-11-08 | 125 | 0.03 | N.A. | 0.02 | 2.21 |
| 78-11-21 | 139 | 0.09 | 0.08 | 0.12 | 0.03 |
| 78-12-19 | 167 | 0.23 | 0.02 | 0.10 | 0.16 |
| 79-01-23 | 202 | N.T. | 0.01 | 0.06 | 0.11 |
| 79-02-20 | 230 | N.T. | 0.07 | 0.03 | 0.04 |
| 79-03-13 | 251 | 0.29 | 0.01 | 0.08 | 0.02 |
| 79-04-24 | 293 | 0.24 | 0.02 | 0.29 | 0.14 |
| 79-05-23 | 322 | 0.14 | N.T. | 0.29 | 0.47 |
| 79-06-20 | 349‡ | 0.10 | N.T. | 0.17 | 0.14 |
| 79-07-24 | 384‡ | 0.36 | 0.01 | 0.445 | 0.92 |

*(Oil+D) is oil and dispersant treatment.
†The weight of oil in a gram of sediment.
‡The values given for this date are the averages of many samples taken on this date.
N.A. – No analysis.
N.T. – No sample taken.

The major effect of the dispersant was to introduce more oil into the water column for a longer period of time compared with the system without the dispersant added. The high concentrations of oil in the oil-dispersant ponds diminished as did the visible surface sheens by about day 70. Oil in the water column could have risen to the surface, have been metabolized by bacteria or have sunk to the sediment. Undoubtedly, some of each action occurred. In the oil-dispersant-treated ponds, the oil in the water column should have spread evenly over the sediment, as the oil was evenly distributed throughout the water. In all of the treated ponds, surface oil, after sufficient aging or weathering, would adhere to the liners or sink in a random fashion, producing areas of high oil concentration.

Within a week, DO levels in both oil-dispersant-treated ponds had decreased to less than 5 ppm. Concurrent with this were large decreases in the zooplankton populations, increases and decreases in the fungal abundance, decreases in the total bacterial biomass and alterations in the phytoplankton communities. Values of DO remained lower in these ponds than in the control and oil-treated ponds until later than 28 days after treatment, at which time the oil concentrations had decreased to about 4 ppm. During this time, high heterotrophic bacterial and slightly elevated geo-aquatic fungal populations were obtained from the water of all of the treated ponds. The oil had a primary effect on the heterotrophic bacteria, as the heterotrophic populations were of similar magnitude and had the same fluctuations as those bacteria that could grow on agar plates where oil was the sole carbon source. As there were no significant differences between the numbers of fungi from the control and treated ponds enumerated on media that contained oil as the sole carbon source, it may be inferred that the fungi in the treated ponds did not preferentially use the oil as the nutrient source. If they were not utilizing the oil, the fungi were apparently using decaying plant and animal tissue, a secondary effect of the oil. The concentration of 1 ppm of oil in the oil-treated pond was sufficient to produce heterotrophic bacterial populations about ten times greater than in the control pond. After day 84, the heterotrophic bacterial recoveries from all treated ponds were similar, as were the oil concentrations. Throughout the winter the treated ponds tended to have higher recoveries of heterotrophs than the control pond.

The phytoplankton biomass in the oil-dispersant-treated ponds was dominated by single species for the first few months after addition of the chemical. Ponds Nos. 1 and 3 had several contributing classes to the phytoplankton by days 69 and 97, respectively. At these times the concentrations of oil in the water columns were less than 2 ppm. Previously, the concentrations of oil as well as the dissolved organic carbon in pond No. 3 experienced little, if any, impact from the added oil, which was less than 2 ppm except for the initial pulse.

Periphyton collected from the plates was similar in all of the ponds at day 28, but at day 55 after treatment,

there were noticeable differences between the oil-dispersant ponds and the control and oiled ponds. During the decrease of the water column oil-dispersant concentrations in the first week after treatment, many of the dispersed oil droplets returned to the surface. During subsequent decreases of the oil-dispersant concentration, some of the oil may have returned to the surface, but considerable oil sank to the sediment. As the plates were positioned near the centre of the ponds, surface oil which usually collected near the liners and later sank would not directly influence the growth on the plates. By day 55, masses of the filamentous algae *Mougeotia* dominated the periphyton on the plates in pond No. 1 and *Oedogonium* dominated the plates in pond No. 3. Between days 28 and 55, the concentration of oil decreased by 2 ppm in both oil-dispersant-treated ponds and decreased by only 1 ppm after that time. Some of the oil reached the sediment. *Mougeotia* and *Oedogonium* are not known to be heterotrophs, but they were associated with the oil collected on the plates producing suitable conditions for these filamentous green algae to proliferate. When strong reducing conditions were observed near the bottoms of the oil-dispersant-treated ponds in March 1979, only *Mougeotia* was recovered. *Oedogonium* was dominant in the periphyton of ponds Nos. 2 and 4 at this time. These strongly reducing conditions possibly were caused by the respiration of the filamentous algae, fungi and bacteria. There were high sulphur bacterial reducing populations in pond No. 1 at this time and in both ponds Nos. 1 and 3 in April of 1979. At the same time high concentrations of nitrate ion were observed in the waters of ponds Nos. 2 and 4, twice the concentrations found in ponds Nos. 1 and 3. These lower nitrate values were probably caused by denitrifying bacteria that existed under the strongly reducing conditions. Bacteria samples taken from pond No. 3 in March of 1979 were taken from the upper level of the two-level sediment bottom and correspond to densities found in ponds Nos. 2 and 4.

The trends of the periphyton biomass may also reflect other conditions existing in the ponds. Once the plates were colonized, the biomass in pond No. 1 was greater than in pond No. 3 until March of 1979. After this time the biomass values from pond No. 1 were similar to those of the control and oil-treated ponds. Periphyton biomass values from pond No. 3 remained high and persisted at that level until the end of the experiment in July. Zooplankton had been re-established in pond No. 1 by May 1979 and pond No. 1 had populations similar to those of the control pond in June and July. Pond No. 3 had much lower populations of zooplankton than ponds Nos. 1, 2 and 4 during this time. The protozoa collected during April, May and June of 1979 were less abundant in pond No. 3 than in pond No. 1, and pond No. 1 had abundances approaching those of the control pond after April 1979. By the last sampling date in July 1979, all of the ponds appeared to have similar protozoan diversities. The higher biomass of the periphyton material in pond No. 1 and the slightly lower concentration of the oil up to April 1979 suggest that there was a better utilization of the oil and hence a faster recovery rate for this pond.

The zoobenthos results should be indicative of conditions in the sediment, the repository of the sunken oil. Tables 7 and 8 appear to indicate that ponds Nos. 3 and 4 have recovered and are healthier than the control ponds and pond No. 1, as they have higher numbers of invertebrates. However, this may not be the case. If only Chironomidea are considered, as they are common to all ponds, the control ponds have very few individuals. This may be the result of the emergence of the Chironomidea prior to the final sampling, leaving low numbers. The few individuals in pond No. 1 had larger volumes than in the other ponds, and these may have been preparing for emergence or have been closer to emergence than those in the other ponds. In pond No. 3 there is a difference in volume pattern, depending on whether the sample was collected from the lower or upper level of this pond. The Chironomidea found in the lower level, which had the same strong reducing conditions as found in pond No. 1, were larger than those taken from the upper level of this pond. Also to be considered is the fact that the average oil concentrations in the sediment at the last sampling date were the highest in pond No. 4 and the lowest in pond No. 1. The large calculated volumes primarily resulted from the width measurements rather than the lengths. If the ponds experienced synchronous egg laying from the adults early in the year, the oil in the sediment may have delayed the hatching of the eggs or caused an increase in the instar duration.

In a previous study (6), varying amounts of Norman Wells crude oil were added to lined-pond ecosystems. It was found that the more oil that was added to the system, the greater the stress on the elements of the biota that were examined. These elements included heterotrophic bacteria whose populations increased, the zooplankton whose populations decreased, and phytoplankton whose composition and populations altered with increasing oil concentrations. In the present study, considerably less oil was used than in the former study. Even at the low concentrations employed, there was a reduction in the zooplankton populations, enhancement of the heterotrophic bacteria and fungal populations, and elimination of surface insects, with a minimal effect on the phytoplankton and an alteration in the protozoan community. The water column biota in the oil-treated pond had recovered by the following

spring. However, over one half of the oil that was initially added was found in the sediment one year after treatment. Chromatograms of this oil were similar to those of water surface oil analyzed in the fall of the previous year. It is unlikely that this sediment oil would be significantly degraded by bacterial action.

The action of the dispersant was to increase the amount of oil in the water column, and the higher oil concentration caused a considerable perturbation, directly or indirectly, to the biota in the system. The heterotrophic bacterial populations were higher than in the oil-treated pond and the fungal populations were also enhanced. The ciliated protozoan community was altered, with all other zooplankters being eliminated. The number of major contributing classes to the phytoplankton community was reduced when the oil concentrations were greater than 2 ppm, and the amount of attached material was greatly increased. Water surface insects were not observed, and the number and types of benthic organisms were reduced. After the following spring, all of the water column components of the biota had recovered and were similar to those of the control pond by July of that year. At the time of the last sampling in July 1979, less than one third of the oil initially added to these two oil-dispersant-treated ponds was found in the sediment.

These studies were conducted to reflect the worst possible case, one in which the oil is contained and the water not exchanged. Such restrictions do not inhibit various life forms from entering the systems, as indicated by the change from lake zooplankton to pond zooplankton during the first winter and as noticed during the recoveries of the ponds. In more open systems where the water is exchanged there would be differences in the impacts. Turbulent diffusion may dilute the dispersed oil in the water column significantly before the oil droplets can surface or settle to the sediment. With sufficient dilution, the emulsions should not affect the plankters. However, if the oil is driven to shore or a littoral zone, many of the effects noted in this report may occur, especially the buildup of periphyton and the effect on zoobenthos, as well as the encouragement of heterotrophic bacterial growth.

# References


1. Environmental Protection Service. 1977. A selected bibliography on the fate and effects of oil relevant to the Canadian marine environment. Economic and Technical Review Report EPS-3-EC-77-23, Fisheries and Environment Canada.

2. Environmental Protection Service. 1981. A bibliography on freshwater oil spills. EPS Internal Report, EE-21.

3. Snow, N.B. and B.F. Scott. 1975. The effect and fate of crude oil spilt onto Arctic lakes. Proc. of Conference on Prevention and Control of Oil Pollution, March 25-27, 1975, San Francisco, p. 527.

4. Hellebust, J.A., B. Hannah, R.G. Sheath, M. Gregor and T.C. Hutchinson. 1975. Experimental crude oil spills on a small sub-arctic lake in the Mackenzie Valley, N.W.T.: Effects on phytoplankton, periphyton and attached vegetation. Proc. of Conference on Prevention and Control of Oil Pollution, March 25-27, 1975, San Francisco, p. 509.

5. Barsdate, R.J. 1972. Ecological changes in Arctic tundra ponds following exposure to crude oil. Proc. of 23rd Alaska Science Conference, Am. Assoc. Adv. Sci., Washington, D.C., p. 118.

6. Scott, B.F. and D.B. Shindler. 1978. Impact of crude oil on planktonic freshwater ecosystems. Proc. of Conference on Assessment of Ecological Impact of Oil Spills, Keystone, Col., p. 713.

7. Shindler, D.B., B.F. Scott and D.B. Carlisle. 1975. Effect of crude oil on populations of bacteria and algae in artificial ponds subject to winter weather and ice formation. Verh. Int. Verein. Limnol. 19: 2138.

8. Nagy, E., B.F. Scott and J. Hart. 1981. The fate of oil and dispersant mixtures in freshwater ecosystems. EPS Report No. EPS-4-EC-81-3.

9. Vollenweider, R.A., ed. 1974. A manual for methods for measuring primary production in aquatic environments. Blackwell Scientific Publications: Oxford, London, Edinburgh, Melbourne.

10. Prescott, G.W. 1951. Algae of the western Great Lakes area, exclusive of desmids and diatoms. Cranbrook Inst. Sci., Michigan.

11. Smith, G.M. 1950. *The Freshwater Algae of the United States*. 2nd ed., McGraw-Hill, New York.

12. Skuja, H. 1958. Grundzuge der Algenflora and Algen-vegetation de Fjeldgegenden um Abisko in Schwedisch-Lappland. Nova. Acta Regiae Soc. Sci. Ups. Ser. IV, 18(3).

13. Les algues d'eau douce. Vol. I. Les algues vertes, 1968. Vol. II. Les algues jaunes et brunes, Chrysophycées, Phéophycées, Xanthophycées et diatomées, 1970. Vol. III. Les algues bleues et rouges, 1972. B.P. Boubée et Cie, Paris.

14. Desikachary, T.V. 1959. *Cyanophyta*. I.C.A.R. Monogr., New Delhi.

15. Hubert-Pestalozzi, G. 1938. *Das Phytoplankton des Süsswasser, Die Binnengewässwe*. 1. Blaualgen, Bakterien, Pilze.
Ibid. 1941. 2(i). Chrysophyceen, Farblose, Flagellaten, Hwrweokonten.
Ibid. 1942. 2(ii). Diatomeen.
Ibid. 1968. 3. Cryptophyceae, Chlorophyceae, Chloromonadophyceae, Dinophyceae, 3 (2 Auflage).
Ibid. 1955. 4. Euglenophyceen.
Ibid. 1961. 5. Volvocales.

16. Aldberg, A., S. Holmgren, A. Nauweck and L. Ramberg. 1975. Fytoplankton, Kompendium för undervisningen limnologie. Lininologiska Institutionen, Uppsala, Sweden.

17. Pasches, A., Fisher and Dena. 1930. Die Süsswasserflora Deutschland, O sterruichs and der Schweiz. Vols. 1-12.

18. Patrick, R. and C.W. Reimer. 1966. The diatoms of the United States, exclusive of Alaska and Hawaii. Monogr. Acad. Nat. Sci. Philadelphia, Vol. I, No. 13.

19. Environment Canada. 1979. *Analytical Methods Manual*. Water Quality Branch, Inland Waters Directorate, Ottawa.

20. Burnison, B.K. 1980. Modified dimethyl sulphoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. Can. J. Fish. Aquatic Sci. 37: 729.

21. Stewart, W.D.P., ed. 1974. Algal physiology and biochemistry. Bot. Monogr., Vol. 10, University of California Press, Berkeley and Los Angeles, p. 584.

22. Hutchingson, G.E. 1967. *A Treatise on Limnology*. Vol. 2, John Wiley & Sons Inc., New York, London, Sydney, p. 406.

23. Dobson, A.N. and W.H. Thomas. 1964. Concentrating plankton in a gentle fashion. Limnol. Oceanogr. 9: 455.

24. Corliss, J.O. 1979. *The Ciliated Protozoa: Characterization, Classification and a Guide to the Literature*. 2nd ed., Pergamon Press, Oxford.

25. Lackey, J.B. 1959. Zooflagellates. *In* Freshwater Biology, W.T. Edmonson (ed.), 2nd ed., Chap. 8.

26. Haney, J.F. and D.J. Hall. 1973. Sugar-coated Daphnia: a preservation technique for Cladocera. Limnol. Oceanogr. 18: 331.

27. Chengolath, R. 1971. Systematics and distribution of limnetic Rotifera in Ontario. M.Sc. Thesis, University of Waterloo.

28. Edmonson, W.T., ed. 1959. *Freshwater Biology*. 2nd. ed., John Wiley & Sons, New York and London.

29. Lutoikova, L.A. 1970. Rotifer fauna SSSR. Subclass Eurotatoria. Nauka, Leningrad.

30. Ruttner-Kolisko, A. 1933. Plankton rotifers, biology and taxonomy. English translation of Die Rotatorien, Die Binnengewässer, Vol. 26 (Pt. 1), Supp. British Freshwater Copepoda, R. Gurney (ed.), R. Soc. London, 1(1).

31. Smith, K. and C.H. Fernando. 1978. A guide to the freshwater calanoid and cyclopoid copepod crustaceans of Ontario. University of Waterloo.

32. Saether, D.A. 1969. Some near-arctic podonominae, diamesinae and orthocladinae (Diptera, Chironomidae). F.R.B. Bulletin 170.

33. Oliver, D.R., D.M. Clymont and M.E. Rousei. 1978. A key to some larvae of Chironomidae (Diptera) from the Mackenzie and Porcupine watershed. Fisheries and Marine Service Tech. Rep. No. 791.

34. Ref. 28, Edmonson, op. cit. p. 808.

35. Trottier, R. 1971. Effect of temperature on the life cycle of *Anex junius* (Odonata: Aeshnideo) in Canada. Can. Entomol. 103: 1671.

36. Glooschenko, V. Personal communication.

37. Dutka, G.J. Methods of microbiological analysis of water, wastewater and sediments. Inland Waters Directorate, Canada Centre for Inland Waters, Burlington, Ontario.

38. Yobin, R.S., J.F. Ryan and B.K. Afghan. 1978. An improved method for the determination of adenosine triphosphate in environmental samples. Water Res. 12: 783.

39. Lui, D.L. and B.J. Dutka. 1972. Bacterial seeding techniques: a novel approach to oil spill problems. Can. Res. Dev., Vol. 5.

40. Sparrow, F.K. 1960. *Aquatic Phycomycetes*. 2nd. ed., University of Michigan Press, Ann Arbor.

41. Sherry, J. Unpub. results.

# Appendix A
# Sediment Analysis

# Sediment Analysis

Particle size distribution of the sediment material was determined by Mr. G.A. Duncan of the Hydraulics Division, NWRI, using standard procedures (1). In Table A-1, the results are given in Psi values, since due to the large number of samples examined by the laboratory, "this scale was developed specifically as a statistical device to permit direct application of conventional statistical practises to sedimentary data" (2). Also included are the "Wentworth Grades" (2), which give the unindoctrinated an appreciation of the physical sizes.

**Table A-1. Sediment Characteristics**

| Psi | Wentworth Grade (mm) | Psi | Wentworth Grade (mm) |
|---|---|---|---|
| -4.0 (2.48)* | 16 | 0.5 (7.35) | |
| -3.5 (2.70) | | 1.0 (10.31) | 0.5 |
| -3.0 (2.81) | 8 | 1.5 (14.89) | |
| -2.5 (3.49) | | 2.0 (11.71) | 0.25 |
| -2.0 (3.09) | 4 | 2.5 (10.94) | |
| -1.5 (3.20) | | 3.0 (7.43) | 0.125 |
| -1.0 (3.11) | 2 | 3.5 (3.77) | |
| -0.5 (3.07) | | 4.0 (5.13) | 0.063 |
| 0.00 (4.53) | 1 | | |

Note: By means of standard techniques, the material was found to have the following composition: gravel: 17.76%; sand: 77.1%; silt and clay: 5.13%.

*Numbers in parentheses are the percent within Psi range.

## REFERENCES


1. Duncan, G.A. and G.G. LaHaie. 1979. Size analysis procedures used in the Sedimentology Laboratory. NWRI Manual. CCIW unpub. ms.

2. Krambein, W.C. and F.J. Littlejohn. 1938. Manual of sedimentary petrography. The Century Earth Science Series, K.F. Mather (ed.), Appleton-Century Crofts, Inc., New York, pp. 84-85.

# Appendix B
# Dominant Phytoplankton Species

**Table B-1. Dominant Phytoplankton Species in Ponds and their Percent Contribution (abundance)**

| Date | Pond No. 1 | | Pond No. 2 | | Pond No. 3 | | Pond No. 4 | |
|---|---|---|---|---|---|---|---|---|
| | Phytoplankton | Percent | Phytoplankton | Percent | Phytoplankton | Percent | Phytoplankton | Percent |
| 78-04-19 | *Uroglena botrys* | 38.6 | *Dinobryon sociale* | 5.2 | *D. sociale* | 75.5 | *D. sociale* | 58.6 |
| (day -77) | *Selenastrum minutum* | 9.1 | *Dictyospbaerium* | | *Rhodomonas minuta* v. | | *R. minuta* v. | |
| | *R. minuta* | 7.6 | *pulchellum* | 4.1 | *nannoplanktonica* | 18.4 | *nannoplanktonica* | 27.1 |
| | *R. minuta* v. | | *Cryptomonas erosa* | 5.2 | *Katablepharis ovalis* | 2.0 | *Chrysidiastrum* | |
| | *nannoplanktonica* | 40.6 | *R. minuta* v. | | *R. minuta* | 2.0 | *catenatum* | 10.0 |
| | | | *nannoplanktonica* | 24.7 | | | *C. erosa* | 4.2 |
| | | | *Peridinum pulvisculus* | 0.7 | | | | |
| 78-05-09 | *R. minuta* v. | | *D. sociale* | 63.6 | *D. sociale* | 98.1 | *D. sociale* | 88.3 |
| (day -56) | *nannoplanktonica* | 9.8 | *R. minuta* | 3.2 | *Gymnodinium fuscum* | 0.6 | *D. pulchellum* | 2.3 |
| | *D. pulchellum* | 21.2 | *K. ovalis* | 4.7 | | | | |
| | *Aphanocapsa elachista* | 13.9 | *G. fuscum* | 2.9 | | | | |
| | *Gymnodinium* sp. | 3.8 | | | | | | |
| 78-05-22 | *Chromulina sphaeridia* | 14.5 | *D. sociale* | 42.9 | *D. sociale* | 18.9 | *D. sociale* | 34.6 |
| (day -42) | *C. minuta* | 17.9 | *Oscillatoria* | | *C. minuta* | 5.9 | *C. minuta* | 55.8 |
| | *D. pulchellum* | 26.1 | *obliqueacuminata* | 34.5 | *K. ovalis* | 2.3 | *Chrysochromulina* | |
| | *K. ovalis* | 15.3 | *R. minuta* v. | | | | *parva* | 2.3 |
| | | | *nannoplanktonica* | 14.6 | | | *U. botrys* | 1.2 |
| | | | *K. ovalis* | 5.4 | | | *R. minuta* | 1.5 |
| 78-06-06 | *C. minuta* | 54.0 | *C. parva* | 21.6 | *C. parva* | 86.1 | *C. minuta* | 69.1 |
| (day -29) | *K. ovalis* | 3.8 | *C. minuta* | 64.7 | *C. minuta* | 12.8 | *R. minuta* v. | |
| | *C. erosa* | 5.2 | *K. ovalis* | 2.9 | *Selenastrum minutum* | 1.0 | *nannoplanktonica* | 19.3 |
| | *R. minuta* v. | | *R. minuta* v. | | | | *C. erosa* | 8.8 |
| | *nannoplanktonica* | 2.8 | *nannoplanktonica* | 2.9 | | | *Elakatothrix gelatinosa* | 6.1 |
| | | | | | | | *Mougeotia viridis* | 5.2 |
| 78-06-22 | *Ochromonas* sp. | 20.7 | *C. parva* | 31.0 | *C. parva* | 15.7 | *Ochromonas* sp. | 55.1 |
| (day -14) | *C. parva* | 5.5 | *C. sphaerica* | 11.6 | *C. sphaerica* | 5.3 | *C. parva* | 5.8 |
| | *Chlorella* sp. | 56.7 | *Chrysococcus* sp. | 42.4 | *Chlorella* sp. | 57.7 | *K. ovalis* | 5.5 |
| | | | *Chlorella* sp. | 10.1 | *S. minutum* | 8.7 | *R. minuta* v. | |
| | | | *E. gelatinosa* | 1.8 | *R. minuta* v. | | *nannoplanktonica* | 6.4 |
| | | | | | *nannoplanktonica* | 3.4 | *E. gelatinosa* | 21.0 |
| 78-06-28 | *C. minuta* | 96.0 | *C. minuta* | 54.3 | *C. minuta* | 37.1 | *C. minuta* | 48.8 |
| (day -7) | *D. sociale* | 2.0 | *C. parva* | 9.1 | *C. parva* | 42.9 | *C. parva* | 27.9 |
| | *R. minuta* v. | | *Ochromonas* sp. | 6.9 | *R. minuta* v. | | *O. sphaeralis* | 8.8 |
| | *nannoplanktonica* | 2.0 | *K. ovalis* | 1.7 | *nannoplanktonica* | 4.9 | *E. viridis* | 6.3 |
| | | | | | *Selenastrum minutum* | 5.6 | | |

Table B-1. Continued

| Date | Pond No. 1 Phytoplankton | Pond No. 1 Percent | Pond No. 2 Phytoplankton | Pond No. 2 Percent | Pond No. 3 Phytoplankton | Pond No. 3 Percent | Pond No. 4 Phytoplankton | Pond No. 4 Percent |
|---|---|---|---|---|---|---|---|---|
| 78-07-05 (day 0) | *Chromulina mikroplankton* | 4.4 | *C. parva* | 40.0 | *C. parva* | 82.2 | *C. parva* | 85.7 |
| | *Ochromonas* sp. | 33.8 | *C. minuta* | 35.0 | *R. minuta* v. *nannoplanktonica* | 3.0 | *R. minuta* v. *nannoplanktonica* | 1.3 |
| | *R. minuta* v. *nannoplanktonica* | 3.2 | *U. botrys* | 2.5 | *K. ovalis* | 1.9 | *E. gelatinosa* | 1.7 |
| | *Mougeotia viridis* | 2.1 | *R. minuta* v. *nannoplanktonica* | 10.0 | *Oocystis lacustris* | 0.7 | *Gymnodinium fuscum* | 0.4 |
| | *Aphanocapsa elachista* | 3.2 | *Synedra ulna* | 2.5 | *Selenastrum minutum* | 8.6 | | |
| | | | *M. viridis* | 2.5 | *E. gelatinosa* | 2.9 | | |
| | | | *E. gelatinosa* | 2.5 | *G. fuscum* | 0.4 | | |
| 78-07-06 (day 1) | *Chromulina minuta* | 97.6 | *C. minuta* | 40.2 | *C. minuta* | 54.2 | *C. minuta* | 25.1 |
| | *E. gelatinosa* | 1.4 | *C. parva* | 13.4 | *Selenastrum minutum* | 20.8 | *C. parva* | 69.1 |
| | *M. viridis* | 0.4 | *Pandorina morum* | 3.9 | *Chlorella* sp. | 20.8 | *E. gelatinosa* | 1.7 |
| | *O. lacustris* | 0.5 | *A. elachista* | 12.0 | *O. lacustris* | 4.2 | *Ulothrix* sp. | 2.9 |
| | | | | | | | *R. minuta* | 0.6 |
| 78-07-19 (day 14) | *Chromulina minuta* | 96.4 | *C. minuta* | 42.8 | *Ochromonas* sp. | 45.1 | *C. minuta* | 86.8 |
| | *Ochromonas sphagnalis* | 1.1 | *Chromulina mikroplankton* | 23.0 | *C. minuta* | 32.7 | *C. mikroplankton* | 4.7 |
| | *K. ovalis* | 1.7 | *C. parva* | 18.3 | *Oscillatoria obliqueacuminata* | 20.2 | *E. gelatinosa* | 0.6 |
| | *Chlamydomonas* sp. | 0.6 | *Chromulina* sp. | 3.3 | | | *Chlamydomonas* sp. | 6.9 |
| | | | *K. ovalis* | 1.8 | | | *Kirchneriella contorta* | 0.8 |
| | | | *M. viridis* | 1.3 | | | | |
| 78-08-02 (day 28) | *C. minuta* | 80.9 | *C. minuta* | 64.1 | *Ochromonas* sp. | 73.1 | *Selenastrum minutum* | 96.5 |
| | *C. parva* | 2.8 | *C. parva* | 11.7 | *C. minuta* | 19.8 | *C. parva* | 2.3 |
| | *Chlamydomonas* sp. | 16.3 | *K. ovalis* | 16.4 | *C. parva* | 1.9 | | |
| | | | *Merismopedia glauca* | 7.8 | *Chlamydomonas* sp. | 1.5 | | |
| | | | | | *K. ovalis* | 2.3 | | |
| 78-08-15 (day 41) | *C. minuta* | 95.1 | *C. minuta* | 53.4 | *Ochromonas ovalis* | 96.7 | *S. minutum* | 70.9 |
| | *C. parva* | 3.3 | *O. ovalis* | 4.0 | *C. minuta* | 7.3 | *Chlamydomonas* sp. | 1.2 |
| | *U. botrys* | 1.2 | *Oocystis submarina* | 28.7 | | | *O. ovalis* | 16.6 |
| | *O. submarina* | 0.8 | *Scenedesmus quadricauda* | 2.4 | | | *Peridinium pulvisculus* | 1.7 |
| | | | *K. ovalis* | 4.5 | | | | |
| 78-08-29 (day 55) | *Chlorella* sp. | 86.7 | *C. parva* | 20.6 | *Oocystis submarina* | 34.4 | *Selenastrum minutum* | 47.3 |
| | *Chlorella* sp. | 8.0 | *Dinobryon sociale* | 20.6 | *Chlorella* sp. | 53.6 | *Chlamydomonas* sp. | 2.8 |
| | *Ochromonas nannos* | 5.7 | *Crucigenia rectangularis* | 13.7 | *Chlamydomonas* sp. | 0.1 | *Merismopedia glauca* | 30.1 |
| | | | *Kirchneriella subsolitaria* | 4.6 | *O. nannos* | 3.0 | *P. pulvisculus* | 4.9 |

Table B-1. Continued

| Date | Pond No. 1 | | Pond No. 2 | | Pond No. 3 | | Pond No. 4 | |
|---|---|---|---|---|---|---|---|---|
| | Phytoplankton | Percent | Phytoplankton | Percent | Phytoplankton | Percent | Phytoplankton | Percent |
| 78-09-12 | *O. nannos* | 78.2 | *S. minutum* | 46.1 | *O. submarina* | 99.4 | *S. minutum* | 16.1 |
| (day 69) | *Chromulina sphaeridia* | 3.2 | *C. rectangularis* | 14.3 | *S. quadricauda* | 0.6 | *S. quadricauda* | 2.8 |
| | | | *Chromulina minuta* | 3.2 | | | *Chlamydomonas* sp. | 3.6 |
| | | | *K. ovalis* | 6.4 | | | *C. minuta* | 9.3 |
| | | | *R. minuta* v. *nannoplanktonica* | 10.3 | | | *Merismopedia glauca* | 51.4 |
| 78-09-26 | | | *U. botrys* | 25.2 | *O. submarina* | 10.8 | *Ankistrodesmus falcatus* | 12.9 |
| (day 83) | | | *S. quadricauda* | 5.4 | *Chlorella* sp. | 86.5 | *C. erosa* | 10.8 |
| | | | *Botryococcus braunii* | 20.7 | *Sphaerellopsis fluviatilis* | 0.9 | *M. glauca* | 10.8 |
| | | | *Selenastrum minutum* | 17.8 | *S. minutum* | 0.9 | *Amphidinium turicense* | 8.6 |
| | | | | | *P. pulvisculus* | 0.9 | | |
| 78-10-10 | *C. erosa* | 45.5 | *D. sociale* | 16.5 | *Chrysidiastrum catenatum* | 74.8 | *C. catenatum* | 12.4 |
| (day 97) | *Ochromonas* sp. | 1.2 | *U. botrys* | 51.6 | *C. minuta* | 1.9 | *A. falcatus* | 37.1 |
| | *Dinobryon sociale* | 14.7 | *S. minutum* | 13.4 | *Chlamydomonas* sp. | 1.9 | *C. erosa* | 24.7 |
| | *G. fuscum* | 22.3 | *C. rectangularis* | 3.1 | *Chlorella* sp. | 18.6 | | |
| | *Chlorella* sp. | 8.0 | | | *P. pulvisculus* | 3.3 | | |
| 78-11-21 | *C. catenatum* | 35.9 | *U. botrys* | 63.6 | *G. fuscum* | 23.5 | *U. botrys* | 17.6 |
| (day 139) | *Uroglena botrys* | 26.2 | *D. sociale* | 12.9 | *S. fluviatilis* | 55.8 | *C. minuta* | 41.9 |
| | *C. minuta* | 2.4 | *C. catenatum* | 1.5 | | | *A. falcatus* | 11.0 |
| | *Selenastrum minutum* | 1.4 | *K. ovalis* | 4.5 | | | *B. braunii* | 7.4 |
| | | | | | | | *K. ovalis* | 13.2 |
| 78-12-19 | *C. catenatum* | 22.0 | *U. botrys* | 86.9 | *Ochromonas* sp. | 9.3 | *U. botrys* | 16.6 |
| (day 167) | *U. botrys* | 23.1 | *C. catenatum* | 7.1 | *C. minuta* | 18.6 | *C. erosa* | 10.0 |
| | *C. minuta* | 50.8 | *R. minuta* v. *nannoplanktonica* | 5.5 | *Amphidinium turicense* | 8.1 | *K. ovalis* | 5.3 |
| | *C. erosa* | 2.59 | *R. minuta* | 1.1 | | | | |
| 79-01-23 | *C. minuta* | 50.4 | *Chlamydomonas* sp. | 40.7 | *S. fluviatilis* | 25.9 | *C. catenatum* | 40.8 |
| (day 202) | *Chromulina* sp. | 5.0 | *K. ovalis* | 5.1 | *Chromulina* sp. | 21.0 | *Chromulina* sp. | 16.3 |
| | *Ochromonas mutabilis* | 15.6 | *C. erosa* | 4.2 | *Ochromonas* sp. | 6.7 | *B. braunii* | 16.3 |
| | *K. ovalis* | 4.25 | *A. elachista* | 40.8 | *C. erosa* | 10.0 | *R. minuta* | 9.2 |
| | *O. obliqueacuminata* | 13.3 | | | | | *C. erosa* | 3.1 |

Table B-1. Continued

| Date | Pond No. 1 | | Pond No. 2 | | Pond No. 3 | | Pond No. 4 | |
|---|---|---|---|---|---|---|---|---|
| | Phytoplankton | Percent | Phytoplankton | Percent | Phytoplankton | Percent | Phytoplankton | Percent |
| 79-02-20 (day 230) | | | *R. minuta* v. *nannoplanktonica* | 10.9 | *Chlamydomonas strobschneideri* | 32.0 | *Pedinimonas minutissima* | 70.9 |
| | | | *R. minuta* | 27.9 | *Microthamnion strictissimum* | 13.2 | *M. strictissimum* | 8.5 |
| | | | *Cryptomonas caudata* | 28.8 | *K. ovalis* | 10.5 | *Chlamydomonas globosa* | 7.3 |
| | | | *K. ovalis* | 9.4 | *C. erosa* | 12.1 | *Chlamydomonas* sp. | 7.3 |
| | | | *P. pulvisculus* | 9.4 | | | | |
| 79-03-13 (day 250) | *Oscillatoria limnetica* | 29.7 | *O. limnetica* | 3.4 | *Katablepharis ovalis* | 19.0 | *Ochromonas pallida* | 3.6 |
| | *O. granularis* | 9.9 | *O. pallida* | 3.7 | *Chlamydomonas* sp. | 19.0 | *O. limnetica* | 38.9 |
| | *C. erosa* | 10.9 | *K. ovalis* | 11.4 | | | *Ochromonas granularis* | 14.1 |
| | | | *R. minuta* | 76.0 | | | *Chromulina elegans* | 9.1 |
| | | | *Botryococcus sudeticus* | 5.5 | | | *Chlamydomonas globosa* | 9.6 |
| 79-04-24 (day 293) | *Oscillatoria limnetica forma* | 42.5 | *Ochromonas miniscula* | 64.4 | *O. limnetica* | 41.8 | *Pandorina morum* | 13.5 |
| | *Chromulina minuta* | 18.7 | *O. limnetica* | 5.1 | *K. ovalis* | 13.7 | *Planctococcus alsius* | 6.1 |
| | *Oscillatoria limnetica* | 17.0 | *C. parva* | 4.7 | *Ochromonas miniscula* | 11.9 | *O. miniscula* | 8.4 |
| | *K. ovalis* | 4.2 | *Gymnodinium ordinatum* | 4.4 | *Chromulina minuta* | 11.9 | *Chryolykos skujai* | 11.0 |
| | *Chromulina elegans* | 3.6 | *C. rectangularis* | 3.7 | *Nitzschia gracilis* | 6.8 | *Gymnodinium varians* | 9.9 |
| | | | | | *R. minuta* | 13.7 | | |
| 79-05-28 (day 321) | *O. limnetica* | 17.0 | *O. miniscula* | 26.4 | *O. limnetica* | 37.0 | *O. limnetica* | 2.8 |
| | *O. limnetica forma* | 43.8 | *C. rectangularis* | 18.5 | *Chromulina sphaeridia* | 18.7 | *Chromulina minuta* | 58.9 |
| | *Planktosphaeria gelatinosa* | 11.4 | *C. marssonii* | 11.7 | *C. marssonii* | 13.8 | *Chlamydomonas globosa* | 8.3 |
| | *C. minuta* | 4.3 | *R. minuta* | 13.1 | *P. morum* | 5.9 | *P. morum* | 8.3 |
| | *Cryptomonas marssonii* | 4.4 | *Scenedesmus obliquos* | 5.7 | *Peridinium pusillum* | 5.2 | *C. marssonii* | 2.6 |
| | | | | | | | *D. sociale americanum* | 3.7 |
| 79-06-19 (day 350) | *O. limnetica* | 12.7 | *C. rectangularis* | 50.4 | *O. limnetica* | 45.2 | *Chromulina minuta* | 26.8 |
| | *O. limnetica forma* | 11.4 | *C. parva* | 23.3 | *O. miniscula* | 9.3 | *O. limnetica* | 10.7 |
| | *P. gelatinosa* | 13.7 | *R. minuta* | 4.0 | *Oscillatoria tenuis* | 8.2 | *A. falcatus* | 9.7 |
| | *Carteria klebsii* | 12.9 | *Scenedesmus denticulatus* | 5.3 | *C. sphaeridia* | 8.7 | *S. obliquus* | 7.9 |
| | *O. miniscula* | 14.1 | *O. miniscula* | 2.7 | *C. marssonii* | 5.6 | *Lyngbya limnetica* | 7.0 |
| 79-07-24 (day 385) | *Ochromonas elegans* | 85.6 | *C. parva* | 41.6 | *Planctococcus alsius* | 48.5 | *C. erosa* | 13.4 |
| | *Oscillatoria tenuis* | 3.7 | *Cyanarcus bamiformis* | 16.6 | *Oscillatoria minuta* | 8.3 | *Chromulina minuta* | 11.0 |
| | *C. erosa* | 2.2 | *S. denticulatus* | 16.4 | *C. erosa* | 5.4 | *C. marssonii* | 8.9 |
| | *Nitzschia gracilis* | 1.5 | *Tetraedron minimum* | 6.4 | *C. marssonii* | 6.5 | *A. falcatus* | 8.2 |
| | *Merismopedia glauca* | 1.6 | *Merismopedia tenuissima* | 4.7 | *R. minuta* | 4.3 | *S. obliquus* | 5.7 |

# Appendix C
# Dominant Phytoplankton of Subsamples

**Table C-1a. Composition of Phytoplankton Subsamples for Species Contributing Greater than 0.00045 g/m$^3$, August 29, 1978**

| Species | Position number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Pond No. 1 | | | | | | | | |
| *Chlorella* sp. A | 0.0048 | 0.002 | 0.006 | 0.005 | 0.002 | 0.003 | 0.002 | 0.002 |
| *Chlorella* sp. B | 0.046 | 0.038 | 0.061 | 0.046 | 0.045 | 0.048 | 0.045 | 0.045 |
| *Scenedesmus quadricauda* | | | | 0.003 | | | | |
| *Ochromonas nannos* | 0.013 | 0.018 | 0.027 | 0.036 | 0.014 | 0.015 | 0.013 | 0.002 |
| *Chromulina minuta* | | | 0.001 | 0.006 | 0.002 | | 0.001 | |
| *Chromulina* sp. B | | | | | | 0.006 | | 0.012 |
| *Chromulina mikroplanktonica* | | | | | | | | 0.002 |
| Pond No. 2 | | | | | | | | |
| *Crucigenia* sp. | 0.159 | 0.023 | 0.027 | 0.013 | 0.132 | x | 0.034 | 0.002 |
| *Scenedesmus quadricauda* | 0.001 | 0.001 | x | | | | | |
| *Kirchneriella subsolitara* | 0.002 | | 0.018 | | | | 0.001 | |
| *Oocystis submarina* | 0.016 | | | | | | | |
| *Mougeotia viridis* | 0.018 | | | | | | | |
| *Tetraedron minimum* | | | | 0.009 | | | | |
| *Elakatothrix gelatinosa* | | | | | | 0.002 | | |
| *Oocystis lacustris* | | | | | | 0.005 | 0.014 | |
| *Chlorella* sp. B | | | | | | | | 0.002 |
| *Dinobryon sociale* | 0.032 | 0.046 | 0.003 | 0.015 | 0.004 | 0.003 | 0.010 | |
| *Uroglena botrys* | 0.003 | | | 0.005 | 0.005 | 0.003 | 0.008 | 0.014 |
| *Ochromonas nannos* | | 0.003 | | | | | | |
| *Chrysochromulina parva* | | 0.001 | | | | | 0.001 | |
| *Cryptomonas erosa* | 0.001 | 0.013 | 0.020 | 0.035 | 0.002 | 0.002 | | |
| *Rhodomonas minuta* | 0.012 | | | | 0.002 | 0.001 | 0.001 | 0.004 |
| *R. minuta* v. *nannoplanktonica* | | 0.019 | 0.016 | 0.008 | | | 0.012 | 0.020 |
| *Katablepharis ovalis* | | 0.001 | 0.004 | 0.002 | 0.002 | | 0.004 | 0.008 |
| *Gymnodinium fuscum* | | 0.008 | | | | | | |
| *Peridinium pulvisculus* | 0.004 | | 0.043 | 0.011 | 0.004 | 0.002 | | |
| Pond No. 3 | | | | | | | | |
| *Oocystis submarina* v. *variab.* | 0.105 | 0.068 | 0.130 | 0.136 | 0.105 | 0.133 | 0.092 | 0.114 |
| *Chlorella* sp. A | 0.164 | 0.113 | 0.136 | 0.178 | 0.118 | 0.122 | 0.152 | 0.121 |
| *Chlorella* sp. B | 0.051 | 0.038 | 0.084 | 0.114 | 0.076 | 0.067 | 0.122 | 0.089 |
| *Chlamydomonas* sp. A | 0.001 | | 0.002 | | 0.005 | | | |
| *Ochromonas nannos* | 0.018 | 0.028 | 0.015 | 0.010 | 0.009 | 0.030 | 0.011 | 0.013 |
| Pond No. 4 | | | | | | | | |
| *Oocystis lacustris* | 0.003 | 0.010 | 0.021 | 0.006 | 0.011 | | | 0.086 |
| *Selenastrum minutum* | 0.003 | 0.001 | 0.001 | 0.008 | 0.001 | 0.003 | | 0.002 |
| *Chlamydomonas* sp. A | 0.001 | | 0.001 | 0.001 | | 0.001 | | 0.001 |
| *Ankistrodesmus falcatus* | 0.001 | | | | | | | |
| *Mougeotia viridis* | | 0.016 | | | | | | |
| *Oocystis submarina* | | | | | | | | 0.003 |
| *Ochromonas nannos* | | | | 0.005 | | | | 0.004 |
| *Chrysamoeba* | | 0.004 | 0.010 | | | 0.010 | | 0.008 |
| *Uroglena botrys* | | | | | 0.005 | 0.001 | | |
| *Cryptomonas erosa* | 0.022 | 0.008 | 0.016 | 0.038 | 0.021 | 0.015 | | 0.018 |
| *Rhodomonas minuta* | | 0.002 | 0.002 | 0.005 | 0.002 | | | 0.008 |
| *Peridinium pulvisculus* | 0.012 | 0.052 | 0.081 | | 0.009 | 0.075 | | 0.132 |
| *Merismopedia punctata* | 0.001 | | | | | | | |
| *M. glauca* | | | | | | 0.020 | | |
| *Achnanthes* sp. | | | | 0.001 | | | | |
| *Nitzschia* sp. | | 0.001 | 0.002 | | | 0.002 | | |

Note: Presence of species is indicated by x.

**Table C-1b. Composition of Phytoplankton Subsamples for Species Contributing Greater than 0.00045 g/m³, July 25, 1979**

| Species | Position number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Pond No. 1 | | | | | | | | |
| *Oocystis solitaria* | 0.064 | 0.047 | 0.052 | 0.016 | 0.056 | 0.045 | 0.028 | 0.063 |
| *Asterococcus limneticus* | 0.031 | 0.020 | 0.024 | 0.014 | 0.031 | 0.021 | 0.018 | 0.022 |
| *Crucigenia rectangularis* | 0.010 | 0.004 | 0.007 | 0.004 | 0.003 | 0.005 | 0.009 | 0.010 |
| *Closterium acutum* | | 0.002 | | 0.002 | | | 0.001 | 0.002 |
| *Tetraedron minimum* | | | 0.001 | | | | | 0.001 |
| *Scenedesmus obliquus* | | 0.001 | 0.001 | 0.001 | | | | 0.001 |
| *S. quadricauda* | 0.001 | | | | | | | |
| *Chlorella pyrenoidosa* | | | 0.001 | | 0.001 | 0.001 | 0.001 | 0.001 |
| *Oocystis borgei* | | | | | | 0.006 | 0.002 | |
| *Ochromonas elegans* | 0.573 | 0.559 | 0.739 | 0.685 | 0.455 | 0.545 | 0.482 | 0.748 |
| *Mallomonas tonsurata* | 0.010 | 0.007 | | | | | | |
| *M. pumilia* | 0.039 | 0.002 | 0.010 | 0.002 | 0.014 | 0.008 | 0.001 | 0.002 |
| *M. acaroides* | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.001 | | 0.002 |
| *Rhizochrysis tetragenia* | 0.016 | 0.016 | 0.016 | 0.014 | 0.026 | 0.016 | 0.015 | 0.018 |
| *Chromulina elegans* | 0.001 | 0.001 | | | 0.002 | 0.002 | 0.002 | 0.003 |
| *Cryptomonas erosa* | 0.300 | 0.467 | 0.334 | 0.467 | 0.228 | 0.499 | 0.293 | 0.423 |
| *C. rostratiformis* | 0.038 | 0.019 | | | 0.005 | | | |
| *C. marssonii* | 0.003 | 0.001 | 0.001 | 0.002 | 0.003 | 0.003 | 0.002 | 0.001 |
| *C. ovata* | | | 0.007 | | | | | |
| *Rhodomonas minuta* | 0.004 | 0.004 | 0.003 | 0.003 | 0.002 | 0.002 | 0.003 | 0.005 |
| *R. lacustris* | 0.004 | 0.002 | 0.003 | 0.005 | 0.018 | 0.002 | 0.002 | 0.003 |
| *Katablepharis ovalis* | | | 0.001 | 0.001 | 0.002 | 0.002 | | |
| *Gymnodinium veris* | 0.298 | 0.083 | 0.208 | 0.141 | 0.138 | 0.089 | 0.105 | 0.011 |
| *G. uberrimum* | 0.095 | 0.047 | 0.071 | 0.071 | 0.047 | 0.047 | | 0.023 |
| *G. varians* | 0.004 | 0.002 | | | 0.002 | | | |
| *G. ordinatum* | | 0.002 | 0.004 | 0.013 | 0.018 | 0.033 | 0.012 | 0.013 |
| *G. sp. A* | 0.078 | 0.240 | 0.156 | 0.269 | 0.065 | 0.131 | 0.140 | 0.405 |
| *Peridinium inconspicum* | 0.014 | 0.027 | | | | | | |
| *P. aciculiferum* | 0.011 | | 0.044 | | | | 0.007 | |
| *Nitzschia gracilis* | 0.079 | 0.072 | 0.077 | 0.080 | 0.075 | 0.079 | 0.082 | 0.011 |
| *N. palea* | 0.006 | 0.004 | 0.006 | 0.006 | 0.001 | 0.001 | 0.001 | 0.002 |
| *N. linearis* | 0.010 | 0.010 | 0.008 | 0.005 | 0.010 | | 0.006 | |
| *Oscillatoria tenuis* | 0.009 | 0.035 | 0.006 | 0.024 | 0.031 | 0.047 | 0.073 | 0.017 |
| *O. limnetica* | 0.002 | 0 002 | 0.006 | 0.004 | 0.002 | 0.002 | 0.002 | 0.003 |
| *O. limnetica* v. *forma* | 0.001 | 0.0020 | 0.003 | 0.004 | | 0.002 | 0.001 | |
| *Merismopedia glauca* | 0.004 | | 0.001 | | 0.001 | 0.004 | 0.001 | |
| *Chroococcus limneticus* | | | 0.006 | | | | | |
| Pond No. 2 | | | | | | | | |
| *Tetraedron minimum* | 0.171 | 0.175 | 0.142 | 0.118 | 0.095 | 0.176 | 0.113 | 0.144 |
| *Cosmarium depressum* | | | | | 0.009 | 0.003 | 0.060 | 0.015 |
| *C. bioculatum* | 0.010 | | | | 0.010 | 0.004 | 0.010 | 0.007 |
| *C. subtumidum* | 0.004 | | 0.006 | 0.001 | | | | |
| *C. margaritatum* | | | | 0.003 | | | | |
| *Crucigenia rectangularis* | 0.018 | 0.015 | 0.009 | | 0.009 | 0.009 | 0.021 | 0.008 |
| *Oocystis solitaria* | 0.013 | 0.013 | 0.007 | 0.009 | 0.008 | 0.005 | 0.016 | 0.015 |
| *O. borgei* | 0.006 | 0.003 | | | 0.002 | | 0.008 | |
| *Scenedesmus bijuga* | 0.013 | 0.014 | 0.014 | 0.003 | | 0.002 | 0.006 | |
| *S. denticulatus* | 0.021 | 0.012 | 0.020 | 0.002 | 0.004 | 0.028 | 0.032 | 0.021 |
| *S. obliquus* | 0.002 | 0.006 | 0.003 | | 0.001 | | | |
| *S. bijuga* v. *irregularis* | 0.001 | 0.001 | | 0.001 | | 0.001 | 0.001 | 0.001 |
| *Chlamydomonas* sp. A | 0.006 | 0.007 | 0.004 | 0.002 | 0.001 | 0.009 | 0.005 | 0.006 |
| *Spondylosium planum* | 0.002 | 0.002 | 0.002 | 0.003 | | 0.002 | | |
| *Asterococcus limneticus* | 0.003 | 0.005 | | | 0.003 | 0.003 | 0.005 | 0.002 |
| *Coelastrum sphaericum* | | 0.007 | 0.002 | | | 0.003 | | |
| *Nephrocytium limneticum* | | | | 0.001 | | 0.001 | | |
| *Selenastrum minutum* | | | | | | | 0.001 | 0.002 |
| *Sphaerocystis schroeteri* | | | | 0.005 | | | | |
| *Ankistrodesmus spiralis* | | | 0.006 | | | | 0.001 | |

**Table C-1b. Continued**

| Species | Position number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| *A. falcatus* | | | | | 0.001 | 0.001 | | 0.001 |
| *Staurastrum paradoxum* | | | | 0.007 | | 0.003 | | |
| *Pectodictyon cubicum* | | | | | 0.004 | | | |
| *Chrysochromulina parva* | 0.054 | 0.055 | 0.042 | 0.046 | 0.005 | 0.051 | 0.055 | 0.053 |
| *Dinobryon sociale* v. *amer.* | 0.006 | 0.014 | 0.005 | 0.012 | 0.004 | 0.000 | 0.016 | 0.010 |
| *D. crenulatum* | 0.001 | 0.002 | 0.001 | 0.001 | 0.003 | 0.001 | 0.003 | 0.001 |
| *D. monad* | 0.010 | 0.015 | 0.011 | | 0.001 | | 0.007 | |
| *Ochromonas elegans* | 0.002 | 0.002 | 0.002 | 0.002 | | | 0.008 | 0.008 |
| *O. miniscula* | 0.001 | | | | | 0.004 | | |
| *Mallomonas producta* | 0.003 | | 0.007 | | | | 0.004 | 0.012 |
| *Chromulina sphaeridia* | | 0.002 | | | | | 0.002 | |
| *C.* sp. A | | | | | | | | 0.001 |
| *Mallomonas acaroides* | | | 0.003 | | | | 0.001 | |
| *Cryptomonas erosa* | 0.097 | 0.105 | 0.117 | 0.157 | 0.077 | 0.097 | 0.217 | 0.141 |
| *C. marssonii* | 0.027 | 0.021 | 0.0190 | 0.007 | 0.013 | 0.018 | 0.017 | 0.013 |
| *C. ovata* | 0.006 | | 0.007 | 0.005 | | | | |
| *C. rostratiformis* | | 0.005 | 0.003 | | | | | |
| *Rhodomonas minuta* | 0.004 | 0.006 | 0.006 | 0.012 | 0.002 | 0.008 | 0.004 | 0.008 |
| *Katablepharis ovalis* | 0.003 | 0.001 | 0.001 | 0.006 | | 0.003 | 0.001 | 0.006 |
| *Peridinium pusillum* | 0.318 | 0.154 | 0.338 | 0.092 | 0.246 | 0.174 | 0.081 | 0.092 |
| *P. aciculiferum* | 0.035 | 0.017 | 0.070 | 0.014 | 0.054 | | | |
| *Gymnodinium uberrimum* | 0.110 | 0.110 | 0.088 | 0.066 | 0.066 | 0.044 | 0.022 | 0.066 |
| *G. ordinatum* | 0.009 | 0.013 | 0.018 | 0.009 | 0.009 | 0.004 | | |
| *G. varians* | 0.001 | | 0.001 | | | | | |
| *Cyanarcus hamiformis* | 0.001 | 0.001 | 0.001 | 0.001 | | 0.001 | 0.001 | 0.002 |
| *Merismopedia tenuissima* | 0.001 | | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| *Oscillatoria limnetica* | | | 0.001 | | | | | |
| Pond No. 3 | | | | | | | | |
| *Plantococcus asilus* | 0.290 | 0.298 | 0.330 | 0.327 | 0.354 | 0.370 | 0.376 | 0.362 |
| *Oocystis solitaria* | 0.009 | 0.000 | 0.008 | 0.013 | 0.015 | 0.010 | 0.008 | 0.009 |
| *Oocystis lacustris* | 0.002 | | | 0.001 | | 0.001 | | |
| *Closterium acutum* | 0.003 | | 0.001 | | | | 0.002 | |
| *Crucigenia rectangularis* | 0.003 | | 0.001 | | | 0.005 | 0.003 | 0.013 |
| *Chlorella pyrenoidosa* | 0.001 | | | | | | | |
| *Sphaerocystis schroeteri* | | 0.005 | 0.004 | 0.002 | | | 0.002 | 0.001 |
| *Mougeotia* sp. A | | 0.004 | | | | | | |
| *Ankistrodesmus spiralis* | | | 0.001 | | | | | |
| *Cosmarium bioculatum* | | | | 0.002 | | 0.005 | | 0.002 |
| *Asterococcus limneticus* | | | | | | 0.002 | | |
| *Pandorina morum* | | | | | | | | 0.006 |
| *Ochromonas globosa* | 0.003 | | 0.002 | | 0.002 | | 0.003 | |
| *O. miniscula* | | 0.001 | 0.003 | 0.008 | 0.006 | 0.001 | 0.001 | |
| *O. sphaeridia* | | | | | | | | 0.002 |
| *Dinobryon monad* | | | 0.001 | | | 0.001 | | |
| *D. campanulostipitum* | 0.001 | 0.001 | | | 0.001 | | | 0.001 |
| *Chromulina sphaeridia* | 0.002 | | | | | | | |
| *C. minuta* | | 0.001 | | | 0.001 | 0.002 | 0.001 | |
| *Rhizochrysis tetragena* | 0.002 | | | | 0.002 | | | |
| *Cryptomonas erosa* | 0.125 | 0.282 | 0.097 | 0.041 | 0.052 | 0.391 | 0.093 | 0.173 |
| *C. marssonii* | 0.020 | 0.013 | 0.016 | 0.078 | 0.041 | 0.019 | 0.037 | 0.021 |
| *C. ovata* | 0.007 | 0.002 | | | | | | |
| *Rhodomonas minuta* | 0.006 | 0.007 | 0.005 | 0.005 | 0.004 | 0.002 | 0.002 | 0.004 |
| *Katablepharis ovalis* | | | 0.001 | | | | | |
| *Gymnodinium uberrimum* | 0.044 | 0.044 | 0.022 | 0.044 | 0.044 | 0.022 | 0.066 | |
| *G. ordinatum* | 0.027 | 0.016 | 0.017 | 0.008 | 0.008 | 0.008 | 0.016 | 0.015 |
| *G. varians* | | | 0.007 | 0.001 | | | | |
| *G.* sp. A | | | | | | | 0.064 | 0.011 |
| *Peridinium pusillum* | 0.014 | | 0.027 | 0.014 | 0.027 | | 0.027 | |
| *P. aciculiferum* | | 0.009 | | | | | | |

**Table C-1b. Continued**

| Species | Position number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| *P. inconspicum* | | | 0.018 | 0.011 | 0.011 | 0.018 | 0.005 | 0.005 |
| *Nitzschia linearis* | 0.021 | 0.036 | 0.016 | 0.038 | 0.038 | 0.021 | 0.014 | 0.031 |
| *N. gracilis* | 0.011 | 0.010 | 0.010 | 0.015 | 0.013 | 0.004 | 0.006 | 0.004 |
| *N. palea* | | | | | 0.002 | | | |
| *Oscillatoria tenuis* | 0.013 | 0.026 | 0.004 | 0.030 | 0.003 | 0.022 | 0.021 | 0.046 |
| *O. limnetica* | 0.003 | 0.002 | 0.001 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
| *Merismopedia glauca* | 0.001 | | | | | | | 0.001 |
| *Lyngbya limnetica* | | | 0.002 | | | | 0.002 | |
| *Aphanizomenon flos-aquae* | | | | | 0.003 | | | |
| Pond No. 4 | | | | | | | | |
| *Chlorella* sp. A | 0.017 | 0.004 | 0.014 | 0.004 | 0.007 | 0.007 | 0.013 | 0.001 |
| *C.* sp. B | 0.001 | | | | | | | |
| *Oocystis solitaria* | 0.014 | 0.011 | 0.016 | 0.012 | 0.014 | 0.015 | 0.010 | 0.017 |
| *O. lacustris* | | | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 |
| *O. submarina* | 0.001 | 0.003 | | | 0.002 | 0.003 | 0.005 | |
| *Ankistrodesmus falcatus* | 0.002 | 0.002 | 0.003 | 0.002 | 0.002 | 0.002 | 0.003 | 0.001 |
| *Cosmarium bioculatum* | 0.002 | 0.001 | | 0.001 | | | | 0.011 |
| *Scenedesmus obliquus* | 0.003 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.004 | 0.001 |
| *S. bijuga* | 0.001 | | 0.001 | | | 0.001 | 0.001 | |
| *S. submarina* v. *variabila* | | | | 0.001 | | | | |
| *Mougeotia* sp. A | 0.002 | 0.001 | 0.005 | | 0.002 | | 0.001 | |
| *Crucigenia rectangularis* | 0.003 | 0.001 | 0.002 | 0.001 | | | 0.001 | 0.001 |
| *Sphaerocystis schroeteri* | 0.002 | 0.001 | | | | | | |
| *Gleocystis planktonica* | 0.001 | 0.001 | | | | | | |
| *Chlamydonomas* sp. A | 0.001 | 0.001 | 0.001 | | 0.001 | | | |
| *C.* sp. B | | | | 0.001 | | | | |
| *Pandorina morum* | | 0.003 | | | | 0.002 | | 0.011 |
| *Plantococcus alsius* | | | 0.001 | | | 0.001 | | |
| *Planktosbraeria gelatinosa* | | | 0.001 | 0.003 | | 0.002 | 0.001 | 0.001 |
| *Cosmarium subtumidum* | | | 0.001 | | | | | |
| *Asterionella limneticus* | | | | 0.001 | 0.001 | | 0.001 | |
| *Coelastrum sphaericum* | | | | | 0.002 | | 0.001 | |
| *Sphaerellopsis cylkindrica* | | | | | | | 0.003 | |
| *Ochromonas miniscula* | 0.008 | 0.004 | 0.022 | 0.005 | 0.008 | 0.013 | 0.020 | 0.001 |
| *O. globosa* | | | 0.003 | | 0.002 | | | |
| *O. elegans* | | | | | | 0.002 | | |
| *Chromulina minuta* | 0.002 | 0.001 | | | | | | |
| *Chrysochromulina parva* | | 0.001 | | | | 0.002 | | |
| *Cryptomonas erosa* | 0.097 | 0.161 | 0.155 | 0.219 | 0.081 | 0.406 | 0.110 | 0.161 |
| *C. marssonii* | 0.017 | 0.006 | 0.013 | 0.007 | 0.018 | 0.017 | 0.025 | 0.008 |
| *C. ovata* | | 0.003 | | | | | 0.006 | |
| *C. curvata* | | | | 0.003 | 0.004 | | | |
| *Katablepharis ovalis* | 0.002 | 0.001 | 0.002 | 0.002 | 0.001 | 0.002 | 0.002 | |
| *Peridinium pusillum* | 0.052 | 0.015 | 0.102 | 0.009 | 0.040 | 0.041 | 0.114 | 0.015 |
| *P. inconspicum* | 0.015 | 0.003 | 0.027 | 0.004 | 0.026 | 0.024 | 0.017 | 0.002 |
| *Gymnodinium ordinatum* | 0.010 | 0.003 | 0.009 | 0.002 | 0.010 | 0.014 | 0.009 | 0.002 |
| *G. varians* | 0.001 | | 0.001 | | | | 0.001 | |
| *G. uberrimum* | | | 0.011 | | 0.015 | 0.015 | 0.018 | 0.007 |

# Appendix D
# Zooplankton Composition

**Table D-1. Species List of Zooplankton in Ponds Nos. 1 to 4 and Relative Abundance According to Sampling Date**

| Species | 78-01-18 | | | | 78-02-07 | | | | 78-02-28 | | | | 78-03-21 | | | | 78-04-19 | | | | 78-05-09 | | | | 78-05-23 | | | | 78-06-06 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| *Polyarthra dolichoptera* | C | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. dolichoptera vulgaris* | C | C | M | M | C | C | M | M | C | C | C | C | C | C | C | M | R | X | X | R | C | C | C | C | X | C | C | M | C | C | C | C |
| *P. major* | | | | | | | | | | | | | | | | | | | | | X | X | C | X | R | R | | X | | | | |
| *P. euryptera* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Keratella cochlearis cochlearis* | | | | | | | X | | R | X | X | | R | X | R | | | R | | | | X | R | | R | C | X | X | R | X | C | |
| *K. cochlearis v. robusta* | R | C | X | X | X | C | X | X | | C | X | | R | X | X | R | | | | | | | | | | | | | | | | |
| *K. earlina* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *K. crassa* | | | R | | | | X | | | | X | | | | X | | X | X | R | | | X | | | | C | C | | | | | |
| *K. quadrata* | | X | | | | X | | | | X | R | | | R | | | | R | | | | | | | | X | R | | | | | |
| *Asplancha* sp. | | | | | X | R | | | | R | | | | | | | | | | | | | | | R | | | | | | | |
| *Lecane luna* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *L.* sp. | | | | | | R | | | | | | | | | | | | | | | | | | | | | | | X | X | X | |
| *Synchaeta* sp. | | | | | | | | | | | | | | | | | | R | | | | R | | | R | | | R | R | | | |
| *Brachionus angularis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *B. urceolaris* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Lapadella patella* | | | | | | | | | | | | | | | | | | | | | R | R | R | R | X | C | X | X | | | | |
| *Testudinella patina* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Filinia terminalis* | | | | | | | | | | | | | | | | | | | | | | | | | R | | | | | | | |
| *Pompholyx sulcata* | | | | | | | | | | | | | | | | | | R | | | | | | | | | | | | | | |
| *Trichocerca multicrinis* | | | | | | | | | | | | | | | | | | | | | | | | | | | R | | | | | |
| *Daphnia schodleri* | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | |
| *D. pulex* | R | X | | | X | X | | X | X | X | | R | | | | | | | | | | | | | X | R | | R | | R | | R |
| *Chydorus sphaericus* | R | C | X | X | X | X | C | X | C | R | X | M | R | R | X | R | | R | | | R | | | R | R | R | R | R | X | C | C | X |
| *Bosmina longirostris* | | | | | | | R | | | | R | | | | | | | | | | | | | | | | | | X | | X | R |
| *Scapholeberis kingi* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X | R | R | X |
| *Alona* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *A. rectangula* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Diaphosoma leuchtenber* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | R | | |
| *Alonella* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Diacyclops bicuspidatus th.* | | | X | R | | R | X | | | | R | | | X | | | | | | | R | | | | | R | R | R | | | R | |
| *Acanthocyclops vernalis* | | | | | | | R | | R | R | R | R | | | | | | | R | | | | | | R | | | R | | | | |
| *Tropocyclops prasinus* | | | | | | | R | R | | | | | | | | | | | | | | | | | | | R | R | | R | X | |
| *Eucyclops serrulatus* | R | R | X | | | | | | R | | | | | | | | | | | | | | | | | | | | | | | |
| *Diaptomus oregonensis* | | | | | | | | | | | | | | | | | R | R | R | | | | | | | | | | | | | |
| *D. minutus* | | R | X | | R | | X | R | X | R | R | R | C | R | X | | | | | | | R | R | | R | R | R | R | | | R | |
| Nauplii | R | X | C | X | X | X | X | X | X | X | X | X | R | X | X | X | R | R | C | R | X | X | C | C | X | C | C | C | C | C | X | C |
| *Chaoborus* sp. | | | | | | | | | | | | | R | | | | | | | | | | | | | R | | R | | | | |

R – rare.
X – 10 or less.
C – less than 100.
M – less than 1000.
P – greater than 1000.

**Table D-1. Continued**

| Species | 78-06-22 | | | | 78-06-28 | | | | 78-07-05 | | | | 78-07-06 | | | | 78-07-12 | | | | 78-07-19 | | | | 78-08-02 | | | | 78-08-15 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| *Polyarthra dolichoptera* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. dolichoptera vulgaris* | C | C | X | M | C | M | C | C | M | M | M | M | C | M | X | M | | C | X | X | X | C | | | | C | R | | | C | | R |
| *P. major* | C | C | C | C | | | | | | | X | X | C | X | X | C | | C | | | | C | | | | C | | | | X | | R |
| *P. euryptera* | | | | | | | | | | | | | | R | | X | | | | | | | | | | | | | | | | |
| *Keratella cochlearis cochlearis* | | | | R | | R | | X | | X | | C | | | | X | | C | | | | X | | | | | | | | C | | |
| *K. cochlearis* v. *robusta* | R | X | | R | | | | | | X | | X | | | | | | | | | | X | | | | C | | | | C | | |
| *K. earlina* | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | |
| *K. crassa* | | | | | | | | R | | | | | | | | X | | R | | | | | | | | | | | | | | |
| *K. quadrata* | | | | | | | | | | | | | R | | | | | | | | R | | | | | | | | | | | X |
| *Asplancha* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Lecane luna* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *L.* sp. | X | R | | R | X | X | | R | | | | | C | | R | X | | | | X | | R | | | | | | R | | | | |
| *Synchaeta* sp. | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Brachionus angularis* | | | | | | | | | | | | | | | | | R | X | | | | | | | | | | | | | | |
| *B. urceolaris* | | | | | | | | | | | | | | | | | R | R | | | | | | | | | | | | | | |
| *Lapadella patella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Testudinella patina* | | R | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Filinia terminalis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pompholyx sulcata* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Trichocerca multicrinis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Daphnia schodleri* | R | R | X | X | R | R | | R | | | | | | | | | | X | | | | | | | | | | | | X | | X |
| *D. pulex* | | | | | | | | | R | X | R | R | | X | | | | | | | | | | | | R | | R | | | | |
| *Chydorus sphaericus* | R | R | R | R | R | R | R | R | | | R | R | | | | | | | | | | | | | | | | | | | | |
| *Bosmina longirostris* | X | R | R | R | R | R | | R | | | M | R | | R | X | | R | X | X | | | | | | | | | | | | | X |
| *Scapholeberis kingi* | R | R | X | R | R | R | R | R | | X | R | X | | | | X | | | | | | | | | | | | | | | | |
| *Alona* sp. | | R | | | | | | | | R | | | | | | | | | | | | | | | | | | | | | | |
| *A. rectangula* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Diaphosoma leuchtenber* | | | | | | | | | | R | R | | | | | | | | | | | R | | | | | | | | | | |
| *Alonella* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Diacyclops bicuspidatus th.* | R | R | R | R | R | X | R | R | X | C | X | X | | | | | | X | | | | | | | | R | | | | | | |
| *Acanthocyclops vernalis* | | | R | | R | R | R | | X | C | R | X | | X | | X | R | | | | | | | | | | | | | | | |
| *Tropocyclops prasinus* | | | | | | R | R | R | | | | | | | | R | | R | | | | | | | | | | | | | | |
| *Eucyclops serrulatus* | R | | R | | | | | | | R | | R | | | | | | R | | | | | | X | | R | | | | | | |
| *Diaptomus oregonensis* | R | R | | | | R | R | R | X | C | R | | | | | | | | | | | | | | | X | | | | R | | |
| *D. minutus* | | | | | | | | | | | | X | | | | | | X | | | | C | | | | | | | | X | | |
| Nauplii | C | C | C | C | X | C | C | C | C | M | C | M | X | C | R | C | | C | | X | | C | | R | R | C | R | X | | C | | X |
| *Chaoborus* sp. | R | | | | R | | | | R | R | R | R | | R | | | R | | | | | | | | | R | | R | | R | | R |

**Table D-1. Continued**

| Species | 78-08-29 | | | | 78-09-12 | | | | 78-09-26 | | | | 78-10-10 | | | | 78-10-24 | | | | 78-11-11 | | | | 78-11-21 | | | | 78-12-19 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| *Polyarthra dolichoptera* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. dolichoptera vulgaris* | R | C | R | R | C | C | X | X | R | M | | | | C | | | R | M | | R | R | M | | M | X | C | R | R | | C | R | R |
| *P. major* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. euryptera* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Keratella cochlearis cochlearis* | | | | | | | | | R | | | | | | | | | | | | | | | | | | | | | | X | X |
| *K. cochlearis* v. *robusta* | | M | | | | M | | R | | M | | R | | M | | | R | M | R | R | R | M | | R | C | P | R | R | | M | R | |
| *K. earlina* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *K. crassa* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *K. quadrata* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Asplancha* sp. | | | | | | | | | | | | R | | | | | | | | | | | | | | | | | | | | |
| *Lecane luna* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *L.* sp. | R | | R | | X | | X | R | R | | R | | | | | | R | | R | R | R | | R | R | R | | R | R | | | | |
| *Synchaeta* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Brachionus angularis* | | | | | | | | | R | | | | | | | | R | | | | | | | | | | | | | | | |
| *B. urceolaris* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Lapadella patella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Testudinella patina* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Filinia terminalis* | | | | | | R | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pompholyx sulcata* | | | | | | | | | R | | | | | | | | | | | | | | | | | | | R | | | | |
| *Trichocerca multicrinis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Daphnia schodleri* | | R | | | | X | | R | | C | | R | | X | | R | R | X | | R | R | X | | R | R | R | | R | | R | | R |
| *D. pulex* | R | | | X | | | R | R | | | | | | | | | | | | | | | | | | | | | | | | |
| *Chydorus sphaericus* | | | | | | | | | | | R | R | | | | | | | | | | | | | | | | | | | | |
| *Bosmina longirostris* | | | | | | | | | | | | | | | R | | | | | | | | | | | | | | | | | |
| *Scapholeberis kingi* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Alona* sp. | | | | | R | | R | R | R | | R | R | R | | | | R | X | R | R | R | | | | | | R | | | R | | |
| *A. rectangula* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Diaphosoma leuchtenber* | | R | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Alonella* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Diacyclops bicuspidatus th* | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | |
| *Acanthocyclops vernalis* | | | | | | | | | | | | | | | | | R | | | | | | | | | | R | | | | | |
| *Tropocyclops prasinus* | | | | | | | | | | | | | | | | | | | | | | R | | | | | | | | | | |
| *Eucyclops serrulatus* | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | |
| *Diaptomus oregonensis* | | | | | X | | | | | X | | | R | | | | | | | | | | | | | | | | | | R | |
| *D. minutus* | | R | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Nauplii | R | C | R | X | R | C | R | X | R | C | R | X | | | | | X | X | R | R | R | X | R | R | R | X | R | | R | | R | |
| *Chaoborus* sp. | R | R | R | R | R | X | R | | | | | | | R | | R | R | | | | | | | | | R | R | | R | | | |

**Table D-1. Continued**

| Species | 79-01-23 | | | | 79-02-02 | | | | 79-03-13 | | | | 79-04-24 | | | | 79-05-23 | | | | 79-06-19 | | | | 79-07-24 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| *Polyarthra dolichoptera* | | | | | | | | | R | | | | R | | R | X | | | | | | | | | | | | |
| *P. dolichoptera vulgaris* | R | R | R | | | X | R | | R | X | | | | X | R | R | C | M | X | R | M | M | R | C | M | M | X | C |
| *P. major* | | | | | | | | | | | | | | | | | | | | | | | | | C | | R | |
| *P. euryptera* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Keratella cochlearis cochlearis* | | X | | R | | C | R | | | X | X | | | R | R | | R | | | | | | | | X | | R | X |
| *K. cochlearis* v. *robusta* | | | | | | | | | | | | | | R | | | | | | | | | | R | | M | | |
| *K. earlina* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *K. crassa* | | | | | | R | | | | | | | | | R | | | R | X | | | R | | | | | | |
| *K. quadrata* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Asplancha* sp. | | | | | | | | | | | | | R | | | | | | | | | | | | | | | |
| *Lecane luna* | | | | | | | | | | | | | | | R | | R | R | X | R | X | | R | R | | | R | R |
| *L.* sp. | R | R | | | | R | | | | | | | | | | | | R | X | R | | | R | R | | R | R | R |
| *Synchaeta* sp. | | | | | | | | | | | | | R | | | | | | | | R | | R | | | | | |
| *Brachionus angularis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *B. urceolaris* | | | | | | | | | | | | | | | | | R | | | | | | | | | | | |
| *Lapadella patella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Testudinella patina* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Filinia terminalis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pompholyx sulcata* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Trichocerca multicrinis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Daphnia schodleri* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *D. pulex* | R | | R | X | | R | R | X | | | | R | | R | | R | X | X | X | R | R | R | R | X | X | R | X | X |
| *Chydorus sphaericus* | | | | R | | R | | | R | X | R | | | R | | | | R | R | | R | R | R | | R | R | R | R |
| *Bosmina longirostris* | | | | | | | | | | | | | | | | | | | R | | | | R | | | | | |
| *Scapholeberis kingi* | | | | | | | | | | | | | | | | | R | R | R | | R | R | X | X | R | R | | R |
| *Alona* sp. | | | | | | | | | | | | | R | | | | | | | | | | | | X | R | X | |
| *A. rectangula* | | | | | | | | | | | | | | | | | | | | | X | | R | | | | | |
| *Diaphosoma leuchtenber* | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Alonella* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Diacyclops bicuspidatus th.* | | | | | | | | | | | R | | R | | | | | R | R | | | X | | | R | R | | R |
| *Acanthocyclops vernalis* | R | | | | | | | | | | | R | | R | | | | | R | | R | | | R | | | | |
| *Tropocyclops prasinus* | | | | | | | | | | | | | | | | | | | R | | R | R | | | X | R | X | |
| *Eucyclops serrulatus* | | | | | | | | | | | | | | | | | | | R | | | R | | | | | | |
| *Diaptomus oregonensis* | | | | | | R | | | | | | | | | | | R | | | | | | | | | R | | |
| *D. minutus* | | | | | | | | | | | | | | | | | | R | | | | R | | | | | | |
| Nauplii | R | R | R | R | | R | R | X | C | X | X | | R | X | R | R | X | M | C | X | C | C | R | X | C | X | C | C |
| *Chaoborus* sp. | | | | | | R | | | | | | | | R | | | | R | | | R | | | | R | | R | |

## Appendix E
## Toxicity Tests for Water on *Spirillum Volutans*

# Toxicity Tests for Water on *Spirillum Volutans*

*Spirillum volutans*, a large aquatic bacterium with a rotating fascicle of flagella at each pole, was used to test the water samples for toxicity following the modification by Dutka (1) of a procedure developed by Bowdre and Krieg (2). These tests were performed on surface water samples collected from all of the ponds, immediately prior to the treatment, and on days 7, 14 and 28 after treatment.

After 120 min, the acute toxicity procedure produced *no observable effects* on the *Spirillum volutans*. Both unconcentrated and x 10 concentrated (produced by flash evaporation at 45°C) samples were used for this test.

## REFERENCES

1. Dutka, B.J., ed. 1978. Methods for microbiological analysis of water, waste waters, and sediments. Department of Fisheries and the Environment, Inland Waters Directorate, Canada Centre for Inland Waters, Burlington, Ontario.

2. Bowdre, J.H. and N.R. Krieg. 1974. Water quality monitoring: bacteria as indicators. Bulletin 69, Virginia Water Resources Research Center, Virginia Polytechnic Institute.

# Appendix F
# Culturing Media for Fungi

# Culturing Media for Fungi

**Table F-1. Constituents of Modified Aureomycin Rose Bengal Glucose Peptone Agar (mARGPA)**

| Constituent | Concentration (g/L distilled $H_2O$) |
|---|---|
| Glucose | 10.0 |
| Peptone | 5.0 |
| $KH_2PO_4$ | 1.0 |
| $MgSO_4$ $7H_2O$ | 0.5 |
| Agar | 20.0 |
| Rose bengal | 0.035 |
| Aureomycin HCl | 0.2 |

## PREPARATION

Soak and dissolve all ingredients except Aureomycin in 800 mL distilled water for 15 min and then bring to the boil to dissolve. Sterilize by autoclaving at 121°C for 15 min and then cool to 42°C to 46°C, pH 5.4 (approximately). Prepare antibiotic solution separately and add to the cooled medium. Dispense in 6- to 8-mL quantities into petri dishes (50 x 10 mm).

**Table F-2. Constituents of Modified Streptomycin Terramycin Malt Extract Agar (mSTMEA)**

| Constituent | Concentration (g/L distilled $H_2O$) |
|---|---|
| Malt extract | 30.0 |
| Peptone | 5.0 |
| Agar | 15.0 |
| Streptomycin | 0.2 |
| Terramycin HCl | 0.2 |

## PREPARATION

Soak and dissolve all ingredients except streptomycin and Terramycin in 800 mL distilled water for 15 min and then bring to the boil to dissolve. Sterilize by autoclaving at 121°C for 15 min and then cool to 42°C to 46°C, pH 5.4 (approximately). Prepare antibiotic solution separately and add to the cooled medium. Dispense in 6- to 8-mL quantities into petri dishes (50 x 10 mm).

**Table F-3. Constituents of Oil Basal Agar (OBA)**

| Constituent | Concentration (g/L distilled $H_2O$) |
|---|---|
| (i) Basal medium | |
| $NH_4NO_3$ | 1.0 |
| $MgSO_4$ $7H_2O$ | 0.5 |
| KCl | 0.1 |
| $CaCl_2$ | 0.1 |
| $FeSO_4$ $7H_2O$ | 0.01 |
| $KH_2PO_4$ | 1.0 |
| Trace metal solution | 1 mL |
| Agar (Oxoid purified) | 20.0 |
| Rose bengal | 0.035 |
| (ii) Trace metal solution | |
| $(NH_4)_6MO_7O_{24}$ $4H_2O$ | 0.1 |
| $Na_2B_4O_7$ $10H_2O$ | 0.09 |
| $ZnSO_4$ $7H_2O$ | 1.0 |
| MnCl $4H_2O$ | 0.06 |
| $CuSO_4$ $5H_2O$ | 0.35 |
| 2NHCl | 2.5 mL |

## PREPARATION

Filter sterilized (0.2 μm) trace metal solution. Add constituents of basal medium (i) to 1 L distilled $H_2O$. Heat to dissolve. Adjust pH to 5.4 and dispense into boiling tubes (22 mL/tube). Autoclave (10 psi/20 min). Cool in water bath to 50°C to 55°C. Add 2 mL of test oil per tube. Add filtered sterilized Aureomycin (200 μg/mL final concentration) to each tube. Mix thoroughly and pour into plastic petri dishes.

**Table F-4. Constituents of PSP Agar**

| Constituent | Concentration (g/L distilled $H_2O$) |
|---|---|
| $Na_2HPO_4$ $12H_2O$ | 0.596 |
| $KH_2PO_4$ | 2.04 |
| Yeast extract (Difco) | 2.0 |
| Glucose | 10.0 |
| Agar | 20.0 |

## PREPARATION

Adjust pH of the above medium to 6.2 and aseptically add 0.5 g/L sodium benzylpenicillin and 0.5 g/L streptomycin sulphate.

**Table F-5. Dilute Salts Solution**

| Stock solution and volume | Components | Weight |
|---|---|---|
| No. 1, 500 mL | $(NH_4)_2HPO_4$ | 66.04 |
| | $KH_2PO_4$ | 68.05 |
| | $K_2HPO_4$ | 87.09 |
| No. 2, 250 mL | $CaCl_2$ $2H_2O$ | 18.38 |
| | $MgCl_2$ $6H_2O$ | 25.42 |

## PREPARATION

Add 0.5 mL of stock solution No. 1 and 0.1 mL of stock solution No. 2 per litre of distilled water. Autoclave at 121°C for 15 min.